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Natural selection and glucocorticoid physiology

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Abstract

Glucocorticoid hormones are considered potent modulators of trade-offs between reproduction and survival. As such, selection should affect glucocorticoid physiology, although relatively little is known about how selection may act on glucocorticoid profiles. In general, the evolution of physiology is less studied and less well understood than morphological or life history traits. Here, we used a long-term data set from a population of mountain white-crowned sparrows to estimate natural selection on glucocorticoid profiles. Our study suggests that survival selection favours higher hormone concentrations for multiple components of glucocorticoid physiology (both baseline and stress-induced glucocorticoid levels). Fecundity selection varies depending on the component of hypothalamic–pituitary–adrenal physiology; greater reproductive output was associated with higher baseline glucocorticoid levels, but lower stress-induced glucocorticoid levels. Additionally, the selection gradient was greater for glucocorticoids than for a morphological trait (wing length). These results support the hypothesis that stress-induced glucocorticoids increase survival over reproduction within a wild population (the CORT-trade-off hypothesis). Taken together, 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.

Introduction

Relatively little is known about selection on physiological traits as compared to morphological and life history traits (Sinervo & DeNardo, 1996; Feder *et al.*, 2000; Kingsolver *et al.*, 2001; Irschick *et al.*, 2008; McGlothlin *et al.*, 2010). Many physiological traits are thought to be intimately tied to critical fitness components, like survival and reproduction. However, physiological systems often regulate multiple traits that may trade off against one another (Ketterson & Nolan, 1999). Thus, selection on physiological traits may be strong or may be constrained by these trade-offs. It is therefore important to understand the strength and mode of selection on physiological traits.

Endocrine systems exert a powerful influence on an array of behavioural and physiological traits and facilitate the ability of organisms to interact appropriately with their environment (Husak *et al.*, 2009; Cohen *et al.*, 2012). As such, patterns of hormone secretion likely reflect selective pressures and can be used to evaluate the strength of selection. The glucocorticoid hormone axis is an ancient trait found across vertebrate taxa (Close *et al.*, 2010). Glucocorticoids regulate metabolic and stress functions, depending on their secreted level. Baseline levels, which exhibit daily and seasonal variation (Romero, 2002), help regulate metabolism and activity levels (Sapolsky *et al.*, 2000) and represent the current state of allostatic load (accumulated challenge) of the animal (McEwen & Wingfield, 2003). Elevated (stress-induced) levels are secreted in response to diverse challenges including agonistic social interactions, encounters with predators, inclement weather and food scarcity (Wingfield *et al.*, 1998). Elevated glucocorticoids can increase food intake, foraging rates and immune function, and decrease growth, parental behaviours and dominance interactions (see Wingfield & Sapolsky, 2003; for review). In light of these effects, glucocorticoids are thought to promote survival-ori-

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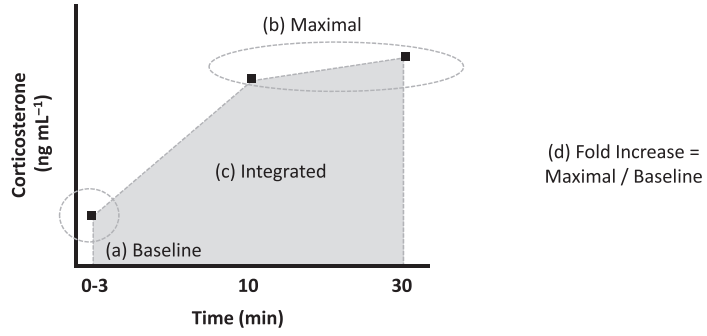
ented physiology and behaviour at the expense of non-critical functions such as reproduction and growth (Wingfield *et al.*, 1998). Thus, much of the endocrine stress literature asserts that acute increases in glucocorticoids mediate fitness trade-offs.

However, the data relating glucocorticoids to fitness are mixed. Three primary hypotheses have been put forth to describe this relationship: the CORT-trade-off hypothesis, the CORT-fitness hypothesis, and the CORT-adaption hypothesis. Under the CORT-trade-off hypothesis, short-term (stress-induced) increases in glucocorticoids are thought to help mediate the trade-off between survival and reproduction by redirecting resources towards survival (Wingfield & Sapolsky, 2003; Almasi *et al.*, 2013). This could create a positive association between glucocorticoid levels and survival and negative association between glucocorticoid levels and reproductive success. Even under this paradigm, glucocorticoids above a certain level likely have a negative impact on survival (Wingfield *et al.*, 1998). Under the 'CORT-fitness hypothesis' (Bonier *et al.*, 2009b), endogenous glucocorticoids should correlate negatively with both reproduction and survival, because environmental challenges should simultaneously elevate glucocorticoids and reduce fitness. An individual that is stressed will have lower fitness because his environment is less optimal than that of a nonstressed individual. Under the CORT-adaption hypothesis (Bonier *et al.*, 2009a), increased glucocorticoids enhance reproduction by facilitating behaviours (such as increased foraging to feed young) that would result in higher reproductive output. For example, elevated CORT is associated with higher foraging intensity and greater food delivery to the chick in Adelie penguins (Angelier *et al.*, 2008). These hypotheses have different underlying assumptions regarding whether comparisons are made within or between individuals, or which measure of glucocorticoids is used. However, they represent the three most prevalent ideas of how glucocorticoids may regulate aspects of fitness. Despite the prevalence of the notion that acute glucocorticoid physiology is related to fitness and the appeal of its logic, relatively few studies have actually measured survival or reproductive success in association with variation in glucocorticoid physiology (Breuner *et al.*, 2008), and none have measured both components of fitness within one population, using appropriate statistical approaches.

Studies of natural selection can 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 (Fig. 1), but physiologists do not agree which elements within the glucocorticoid pathway are most biologically relevant. 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 has a greater biological effect (Breuner & Orchinik, 2002; Romero, 2002; Breuner *et al.*, 2013). Comparing the association of these elements of glucocorticoid physiology with fitness, components may improve our understanding 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 measures of



Measure of Corticosterone	How Measured	Reflects
(a) Baseline	CORT concentration at minute 0-3	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 experience 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 normal course of life (baseline concentrations), which set receptor levels

Fig. 1 An illustration of corticosterone secretion from standardized capture and handling stress protocol. (a) Baseline corticosterone. (b–d) Stress-induced corticosterone.

fitness. We evaluated these relationships from both evolutionary and physiological perspectives. From the evolutionary perspective, we sought to understand the presence and patterns of selection (i.e. mode, direction and strength). From the physiological perspective, we sought 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 adult survival probability (estimated from capture-mark-recapture data).

Materials and 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; approximately 3030 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 (*Salix* spp.) and scrub pine (*Tinus contorta*), which provide nesting sites for the sparrows. Approximately 50 pairs breed at the study site each year. Males arrive at the breeding ground first and females arrive approximately 10 days later on average (Morton, 2002). In a typical year, the birds arrive to find the breeding ground covered with snow and must wait for the snow to recede from nest sites before they initiate nesting (range of clutch initiation date during the study: May 31–June 13). 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. Traps were checked roughly every 60 min. 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 3–May 15). In the years 2001–2006, trapping effort continued through late July (range: July 26–July 31). In 2007 and 2008, trapping effort ceased on May 31 and June 6 respectively. Although trapping effort continued into the nesting period from 2001 to 2006, only blood samples drawn prior to clutch initiation (i.e. during the arrival phase) were included in our study. We limited samples to the arrival phase because corticosterone (the primary avian glucocorticoid) physiology may change as the breeding season progresses (Romero & Wingfield, 1999), and the majority of our samples were taken in the early season. Trapping continued after the arrival phase to inform our adult survival estimates. The habitat was searched to locate as many nests as possible from 2001 to 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.

Blood sampling protocol and analysis

To evaluate organisms' hormonal response to stress, physiologists typically apply a standardized stress protocol and take repeated measures of the resultant glucocorticoid levels (Fig. 1; Wingfield, 1984). The first sample taken within 3 min of disturbance represents circulating levels of glucocorticoids naturally occurring in the animal. They may be elevated if the sparrow is experiencing challenge such as predator pressure or social stress. These baseline samples are thought to represent the current 'allostatic state' of the animal (McEwen, 1998; McEwen & Wingfield, 2003). The serial samples taken after capture represent the potential of the hypothalamic–pituitary–adrenal (HPA) axis to respond to stress. As researchers, we activate the HPA axis with a standard capture and handling protocol, allowing for comparison of HPA reactivity among individuals. These stress-induced levels are quantified as the maximal response glucocorticoid concentration, integrated glucocorticoid concentration and fold-increase in glucocorticoid from baseline to maximal response concentration, but no consensus has emerged as to the most relevant measure (Romero, 2004).

To assess both baseline and stress-induced corticosterone, we took a series of blood samples after capture. An initial blood sample was taken from captured birds immediately upon removal from the trap (within 3 min of disturbance, as per Romero & Reed (2005)) and the interval between trap disturbance and completion of the sample was recorded. Romero & Reed (2005) demonstrated that baseline CORT levels in white-crowned sparrows were not affected by sitting in a Potter trap for 15 min prior to sampling. However, we visited traps approximately every 60 min, so birds could have been in traps for longer than 15 min. White-crowned sparrows do not evidence a change in baseline corticosterone during daylight hours. Levels rise overnight to a peak at the end of the night, but are low and constant throughout the day (Breuner, Wingfield and Romero, 1999). Thus, we were confident comparing samples from across the day. We evaluated baseline CORT levels in a subset of birds (135 samples taken before egg lay in 2006) as a function of time since the trap was last checked. Baseline CORT levels are not predicted by the amount of time since the trap was last checked (linear regression, $F_{1,133} = 0.89$, $P = 0.35$), suggesting that a longer wait time in the trap does not increase CORT secretion in these sparrows. After the initial blood sample was taken, birds were placed in cloth bags as part of the stress protocol and subsequent blood samples were drawn at 15 and 30 min postdisturbance (Fig. 1). Blood samples were

taken by puncturing the brachial vein with a 26-gauge needle and collecting approximately 40–60 μL of blood in a heparinized microcapillary tube. Blood samples were kept in a portable cooler with several icepacks. Within 6 h, microcapillary tubes were centrifuged at 15 000 *g* for 10 min and plasma was removed with a Hamilton syringe and then stored at −20 °C until assayed.

The serial samples allow us to measure multiple aspects of glucocorticoid physiology. Baseline corticosterone (endogenous levels at the time of capture) was measured as the concentration of corticosterone in the initial blood sample.

Stress-induced corticosterone (measuring the potential of the HPA axis to secrete CORT in response to stress) was quantified in three ways (Fig. 1):

- Maximal corticosterone response is the highest concentration of corticosterone measured over the sampling period.
- Integrated corticosterone estimates the total amount of corticosterone secreted over the sampling period by integrating of hormone values across the entire 30 min.
- Fold increase in corticosterone is the maximal response concentration divided by the baseline concentration.

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

Hormone and binding protein assays

Plasma corticosterone concentrations were measured using Enzyme Immunoassay kits (cat # ADI-901-097; Enzo Life Sciences, Plymouth Meeting, PA, USA) 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. Baseline corticosterone samples > 2 standard deviations above the mean were excluded from our analyses, because these individuals likely experienced a stressor directly prior to our sampling (21 out of 617 samples were discarded).

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 may interfere with the ability of circulating glucocorticoids to bind with intracellular receptors in target tissues (Mendel 1989). This is the basis of the 'free hormone hypothesis', which states that the unbound ('free') fraction of glucocorticoids in the plasma is more biologically relevant than total glucocorticoid levels (Breuner *et al.*, 2013). Alternate views of CBG suggest that it may facilitate glucocorticoid action by functioning as a carrier molecule analogous to haemoglobin (Romero, 2002) or by binding to membrane CBG receptors to enable the cellular uptake of glucocorticoids (Breuner & Orchinik, 2002; Malisch & Breuner, 2010).

Plasma CBG capacity was measured using a ligand-binding assay with tritiated corticosterone following an established protocol optimized for white-crowned sparrows (Breuner *et al.*, 2003). All samples were run in triplicate. Assay tubes contained 50 μL of 1 : 300 diluted, stripped plasma, 50 μL [3H] corticosterone and either 50 μL of 1 μM unlabelled corticosterone (nonspecific binding) or 50 mM (pH 7.40) Tris assay buffer (total binding). Final plasma dilution was 1 : 900. 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, USA) over 1 μm binder-free glass mircofibre filters (GF/B; Whatman, Piscataway, NJ, USA) soaked in 25 mM Tris buffer with 3% polyethylenimine for 1 h. 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 & Baumann (1989):

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

where Ka is 1/Kd (nM), 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).

Analysis of survival probability vs. glucocorticoid physiology

Interannual survival probabilities were estimated using the 'recaptures only' model in Program MARK version 6.0. This is a maximum likelihood analysis that decomposes encounter histories (individual presence/absence status for each year) into interannual survival accounting for encounter probabilities (Cooch & White, 2010). This approach takes advantage of 'gaps' in an individuals' encounter history to estimate the encounter probability (e.g. an individual observed in years 1 and 3 has a 'gap' in year 2 where it was alive but not encountered). In our study, an encounter is synonymous with trapping an individual. Survival probability then can be estimated, because the probability of resighting an individual (observed return rate) is the product of that individual surviving and the probability of it being encountered (Cooch & White, 2010). Once survival probability has been estimated, Program Mark can be used to assess whether individual covariates (e.g. mass

or hormone level) are meaningful predictors of survival by evaluating if their inclusion improves the fit of the model.

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. The restriction was enforced due to the itinerant nature of newly arriving birds; these sparrows often explore multiple potential breeding areas before settling down in a single place (Morton, 2002; C. Breuner, personal observation). Including transient individuals can invalidate the survival estimates in capture–recapture models (Pradel *et al.*, 1997). A variety of morphological and glucocorticoid individual covariates were assessed as predictors of survival probability (see below). Some individuals were captured and bled in multiple years. Program MARK cannot readily accommodate these so-called 'individually time-varying covariates' (multiple years per individual, with a hormone value associated with each year). We therefore averaged the corticosterone values across years for individuals with repeated measurements to arrive at a single corticosterone value per individual. Repeatability of the trait varied widely, depending on the measure of glucocorticoid (e.g. baseline vs. maximal corticosterone response). Repeatability describes the degree of variation across individuals vs. the degree of variation within individuals, with higher values indicating that the character trait is more a property of the individual (Lessells & Boag, 1987) Repeatability, r, is defined as

$$r = \frac{s_A^2}{s^2 + s_A^2},$$

where s_A^2 is the among individual variance component and s^2 is the within individual variance component (Lessells & Boag, 1987). Repeatability ranged from 0.10 to 0.37, with an average repeatability of 0.29 (total baseline CORT: $r = 0.104$, $F_{174,445} = 1.416$, $P = 0.002$, $n = 620$; free baseline CORT: $r = 0.216$, $F_{169,405} = 1.94$, $P \leq 0.001$, $n = 575$; total max CORT: $r = 0.369$, $F_{108,199} = 2.68$, $P \leq 0.001$, $n = 308$; free max CORT: $r = 0.369$, $F_{103,190}$, $P \leq 0.001$, $n = 294$; total integrated CORT: $r = 0.360$, $F_{100,186}$, $P \leq 0.001$, $n = 287$; free integrated CORT: $r = 0.319$, $F_{102,181}$, $P \leq 0.001$, $n = 284$). These values are well within range of repeatability estimates for physiological measures (Wolak *et al.*, 2011).

For some individuals, we only had measures of baseline corticosterone. Therefore, we analysed measures of baseline ($n = 207$) and stress-induced ($n = 181$) corticosterone with separate data sets to maximize sample sizes. For each data set, we separately fit and compared the relative support for alternative models using an information theoretical approach (quasi Akaike information criterion, QAIC; Burnham & Anderson, 2002). We first found the best general model (no individual covariates) for each data set by comparing all permutations of models containing effects of year and sex on survival probability and effects of year, sex and trapping effort (number of trap days) on encounter probability. We also investigated models with no main effects on survival, encounter probability or both. General models provided a standard for comparison of models with individual covariates. For individuals with measures of baseline corticosterone, we fit models where survival was a function of baseline total and free corticosterone, mass, wing length or body condition. For individuals with measures of stress-induced corticosterone, we fit models where survival was a function of maximal response, integrated, fold-increase for total and free corticosterone, mass, wing length or body condition.

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:

$$\widehat{M_i} = M_i \left[\frac{L_0}{L_i}\right]^{b_{\mathrm{SMA}}}$$

where M_i and L_i are each individual's respective mass and linear body measurements (here, 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 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). Years of 2001, 2007 and 2008 had too few capture events to include in the corticosterone-survival analysis by year. Their inclusion blocked the model from converging.

Finally, we explored additional models wherein we truncated our data set to exclude portions of corticosterone parameter space with sparse data, because we were concerned that rare high values might 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 present only the more inclusive data set.

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 adhered 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, 2002) and program MARK uses $\hat{c}$ to adjust the AICc using quasi-likelihoods (Anderson & Burnham, 1999). We estimated $\hat{c}$ by dividing the mean deviance and the mean dispersion parameter from bootstrap simulations by the corresponding observed deviance and observed dispersion parameter from the general model (Cooch & White, 2010). We used the higher of these values from each general model as our $\hat{c}$. Goodness-of-fit methods do not exist for models with individual covariates, so we used the $\hat{c}$ from the corresponding general model for our models with covariates (Cooch & White, 2010).

Analysis of reproductive success vs. glucocorticoid physiology

The number of successfully fledged offspring was quantified as the number of offspring last seen in a nest before fledging minus any dead offspring found in the nests after fledging. Nests were monitored every other day. Predated nests were easily identified based on nest condition (predated nests have disturbed nest floors from predation event, whereas successful nests typically have a flattened nest edge where the nestlings departed). Additionally, parents feeding fledglings confirmed successful fledging, whereas rapid re-nesting supported inferences of nest failure.

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 standard regression analysis (Lande & Arnold, 1983; Brodie *et al.*, 1995). Briefly, we fit regressions using relative reproductive success (individual offspring fledged/population mean offspring fledged) as our dependent variables and standardized values for each covariate ((individual value − population mean value)/population standard deviation) as our independent variables. We analysed each sex separately. We also compared reproductive success between first time breeders and returning breeders, because returning breeders often have greater reproductive output (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 analysed these groups separately. All statistical analyses for reproductive success were done using 'R' version 2.11.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

Results

Survival

Baseline corticosterone

In the best general model (without hormonal or morphological predictors), survival probability was independent of sex and year, whereas encounter probability was a function of sex and trapping effort. The model fit improved when total or free baseline corticosterone was included as a covariate of survival probability (Table 1). In these models, higher levels of CORT were associated with greater survival probability. In the best supported models, there was a positive, curvilinear relationship between survival probability and free CORT (Fig. 2a) or total CORT (Fig. 2b). For the years 2002–2006, we allowed the relationship between corticosterone

Table 1 Model selection for baseline CORT and survival; results for the 'recaptures only' model in Program Mark.

Model			Delta	QAICc	Model	No.	
Survivor (ϕ)	Capture (p)	QAICc	QAICc	Weight	likelihood	par	*Q* Deviance
Nat Log (Free)	Sex + Effort	632.15	0	0.24	1	5	622.03
Free + Free^2	Sex + Effort	632.73	0.58	0.18	0.75	6	620.56
Nat Log (Total)	Sex + Effort	632.80	0.65	0.17	0.72	5	622.68
Total	Sex + Effort	633.70	1.55	0.11	0.46	5	623.58
Total + Total^2	Sex + Effort	633.80	1.65	0.11	0.44	6	621.63
Free	Sex + Effort	634.27	2.12	0.08	0.35	5	624.15
Null	Sex + Effort	**635.47**	**3.32**	**0.05**	**0.19**	**4**	**627.39**
Mass	Sex + Effort	636.95	4.80	0.02	0.09	5	626.83
Scaled Mass Index	Sex + Effort	637.14	4.98	0.02	0.08	5	627.02
Wing	Sex + Effort	637.43	5.28	0.02	0.07	5	627.31

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). QAICc is the quasi Akaike Information Criterion, corrected for finite sample sizes. Delta QAICc is the difference between the QAICc for a given model and the QAICc for the best supported model.

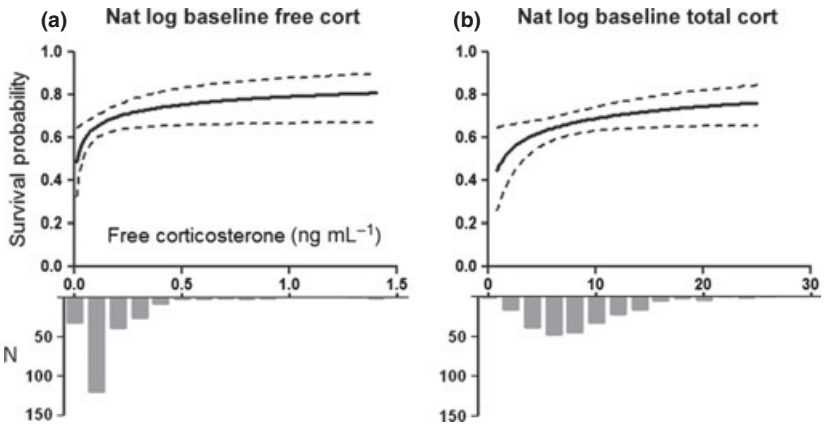


Fig. 2 Relationship between baseline corticosterone (ln log) and survival probability. (a) Best model for baseline free corticosterone. (b) Best model for baseline total corticosterone. Histograms represent the number of individuals in each bin.

and survival probability to vary by year (corticosterone × year interaction), but these models had less support than the general model (Table 2).

Stress-induced corticosterone

In the best general model, survival probability was independent of sex and year, whereas encounter probability was a function of sex and trapping effort. The general model was better supported than all but three models in which survival probability was a function of stress-induced corticosterone (Table 3). For all of these models, higher levels of stress-induced CORT were associated with greater survival probability. In these models, survival was a function of maximal corticosterone response (Fig. 3a,c) or integrated free corticosterone (Fig. 3b). For the years 2002–2006, we again allowed the relationship between corticosterone and survival probability to vary by year (corticosterone × year interaction). For maximal response (Fig. 4) and integrated free corticosterone, this improved the model fit (Table 4).

Reproductive success

All significant relationships between individual covariates and reproductive success were found in returning female breeders and not in first-year females or adult males. Greater reproductive success was observed

Table 2 Model selection for baseline CORT and survival by year; results for the 'recaptures only' model in Program Mark.

Model			Delta	QAICc	Model	No.	
Survival (ϕ)	Capture (p)	QAICc	QAICc	Weight	likelihood	par	Q Deviance
Sex + Nat Log (Total)	Sex	309.32	0	0.27	1	5	299.14
Sex + Nat Log (Free)	Sex	309.64	0.31	0.23	0.86	5	299.46
Sex + Free	Sex	309.83	0.51	0.21	0.78	5	299.66
Null	Sex	**312.91**	**3.56**	**0.04**	**0.17**	**4**	**304.79**
Sex + SMI	Sex	312.93	3.61	0.04	0.17	5	302.75
Sex + Wing	Sex	313.17	3.85	0.04	0.15	5	302.10
Sex + Free × Yr	Sex	313.43	4.11	0.03	0.13	8	296.10
Sex + Nat Log (Free) × Yr	Sex	313.47	4.14	0.03	0.13	8	297.03
Sex + Total × Yr	Sex	313.48	4.15	0.03	0.13	8	297.04
Sex + Nat Log (Total) × Yr	Sex	313.53	4.21	0.03	0.12	8	297.10
Sex + Mass	Sex	314.96	5.64	0.02	0.06	5	304.78
Sex + SMI × Yr	Sex	316.49	7.17	0.01	0.03	8	304.78
Yr	Sex	316.69	7.37	0.01	0.03	6	304.44
Sex + Free × Yr	Sex	317.41	8.09	0.01	0.02	12	292.47
Sex + Free + Free^2 × Yr	Sex	317.48	8.16	0.00	0.02	12	292.54
Sex + Total × Yr	Sex	318.12	8.79	0.00	0.01	12	293.17
Sex + Mass × Yr	Sex	318.65	9.33	0.00	0.01	8	302.22

Survival probability was estimated as 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. QAICc is the quasi Akaike Information Criterion, corrected for finite sample sizes. Delta QAICc is the difference between the QAICc for a given model and the QAICc for the best supported model.

Table 3 Model selection for stress-induced CORT and survival; results for the 'recaptures only' model in Program Mark.

Model							
Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model likelihood	No. par	Q Deviance
Nat Log (Free Max)	Sex + Effort	586.38	0	0.18	1	5	576.24
Nat Log (Free Integr)	Sex + Effort	586.74	0.37	0.15	0.83	5	576.61
Free Max	Sex + Effort	587.15	0.77	0.12	0.68	5	577.02
Null	Sex + Effort	**588.42**	**2.04**	**0.06**	**0.36**	**4**	**580.33**
Free Integr	Sex + Effort	588.43	2.05	0.06	0.36	5	578.29
Free Max + Free Max^2	Sex + Effort	588.61	2.23	0.06	0.33	5	578.47
Total Fold Incr + Total Fold^2	Sex + Effort	589.41	3.07	0.04	0.22	5	579.31
Nat Log (Free Fold Increase)	Sex + Effort	589.74	3.37	0.03	0.19	5	579.61
Free Fold Incr	Sex + Effort	589.80	3.42	0.03	0.18	5	579.67
Free Integr + Free Integr^2	Sex + Effort	589.87	3.49	0.03	0.18	5	579.73
Total Integr + Total Integr^2	Sex + Effort	890.15	3.78	0.03	0.15	5	580.02
Nat Log (Total Fold Incr)	Sex + Effort	590.21	3.84	0.03	0.15	5	580.08
Total Integr	Sex + Effort	590.22	3.85	0.03	0.15	5	580.09
Nat Log (Total Integr)	Sex + Effort	590.23	3.85	0.03	0.15	5	580.09
Total Max + Total Max^2	Sex + Effort	590.29	3.91	0.03	0.14	5	580.16
Nat Log (Total Max)	Sex + Effort	590.34	3.96	0.03	0.14	5	580.21
Total Max	Sex + Effort	590.34	3.97	0.03	0.14	5	580.21
Free Fold Incr	Sex + Effort	590.38	4.00	0.02	0.14	5	580.24
Free Incr + Free Fold Incr^2	Sex + Effort	590.45	4.08	0.02	0.13	5	580.32

Survival was a function of maximal total corticosterone response (Total Max), integrated total corticosterone (Total Integr), fold-increase in total corticosterone (Total Fold Incr), maximal free corticosterone response (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). QAICc is the quasi Akaike Information Criterion, corrected for finite sample sizes. Delta QAICc is the difference between the QAICc for a given model and the QAICc for the best supported model.

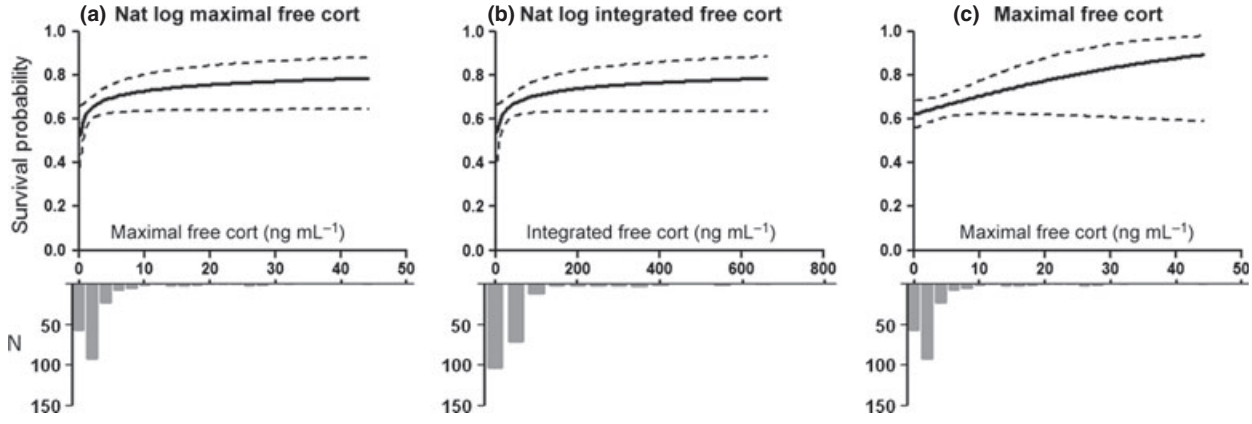


Fig. 3 Relationship between stress-induced corticosterone (ln log) and survival probability for models with lower quasi Akaike information criterion scores than general model. (a) Survival as a function of natural log-transformed free corticosterone. (b) Survival as a function of natural log-transformed integrated corticosterone. (c) Survival as a function of free corticosterone. Histograms represent the number of individuals in each bin.

among returning females with greater baseline total corticosterone (LM: $F_{1,25} = 9.03$, $P = 0.006$; Table 5, Fig. 5a), lower fold-increase for total corticosterone (a smaller proportional increase above baseline corticosterone; LM: $F_{1,21} = 8.62$, $P = 0.008$, Table 5, Fig. 5b) and longer wings (LM: $F_{1,22} = 7.53$, $P = 0.012$; Table 5, Fig. 5c). Wing length, baseline total corticosterone and fold-increase in total corticosterone were significantly associated with reproductive successes with linear selection gradients ranging from −0.18 to 0.14 (Table 5).

We also found two significant quadratic selection gradients among returning female breeders. Females with

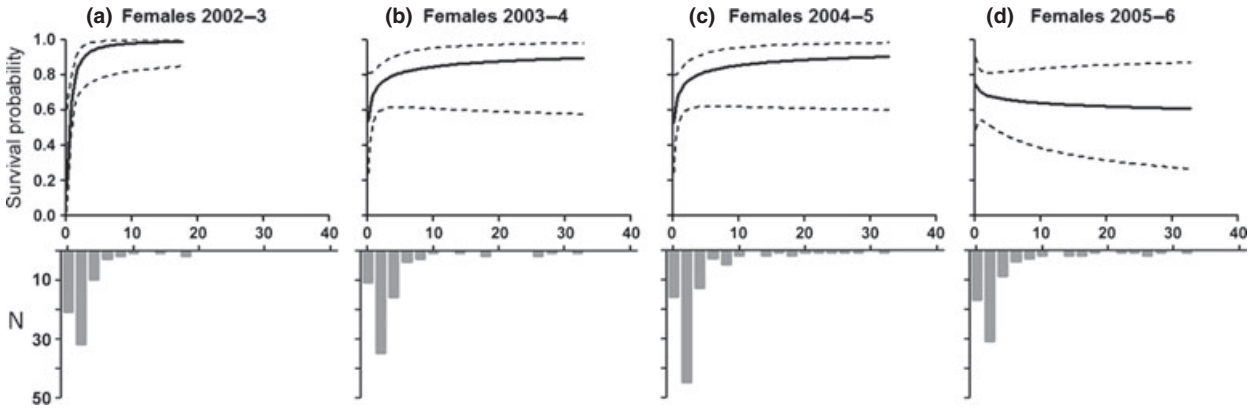


Fig. 4 Relationship in females between maximal free corticosterone response and survival probability where the survival probability is a function of sex and the interaction of year and natural log-transformed maximal free corticosterone response. Histograms represent the number of individuals in each bin.

Table 4 Model selection for stress-induced CORT and survival by year; results for the 'recaptures only' model in Program Mark.

Model							
Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model likelihood	No. par	Q Deviance
Sex + Nat Log (Free Max) × Yr	Sex	369.15	0	0.75	1	8	352.69
Sex + Yr + Nat Log (Free Integr) × Yr	Sex	371.92	2.77	0.19	0.25	11	349.07
Sex	Sex	**375.99**	**6.84**	**0.03**	**0.03**	**4**	**367.87**
Sex + Nat Log (Total Integr) × Yr	Sex	376.49	7.34	0.02	0.03	8	360.03
Sex + Nat Log (Total Max) × Yr	Sex	378.04	8.89	0.01	0.01	8	361.58
Sex + Yr + Nat Log (Free Fold) × Yr	Sex	378.58	9.43	0.01	0.01	11	355.73
Sex + Nat Log (Total Fold) × Yr	Sex	380.96	11.81	0.00	0.00	8	364.50

Survival was a function of maximal total corticosterone response (Total Max), integrated total corticosterone (Total Integr), fold-increase in total corticosterone (Total Fold Incr), maximal free corticosterone response (Free Max), integrated free corticosterone (Free Integr), fold-increase in free corticosterone (Total Free Incr), year (Yr), sex (Sex), mass, body condition (Scaled Mass Index), or no individual covariates (Null, in bold). Resight probability was a function of sex. QAICc is the quasi Akaike Information Criterion, corrected for finite sample sizes. Delta QAICc is the difference between the QAICc for a given model and the QAICc for the best supported model.

intermediate fold-increase in free corticosterone had lower reproductive success (LM: $t_{19} = 2.28$, $P = 0.034$; Table 6, Fig. 5e). Also, females with intermediate fat scores had greater reproductive success (LM: $t_{27} = 2.80$, $P = 0.009$; Supplement: Table 6, Fig. 5b). Quadratic selection gradients for these traits were 0.09 and −0.11, respectively (Table 6).

Discussion

In this study, we set out to describe relationships between fitness components and glucocorticoid physiology, evaluating this relationship from both evolutionary and physiological perspectives. Overall, our results support relationships between corticosterone levels and survival probability, suggesting that survival selection operates on glucocorticoid physiology. Our results also show significant relationships between corticosterone and reproductive success, suggesting that fecundity selection operates on glucocorticoid physiology in females. We acknowledge, however, that the causality of these associations cannot be assessed in a correlational study, such as this one. Therefore, we cannot exclude the possibility that behaviours associated with survival or reproductive success are driving corticosterone physiology.

Survival selection

For survival, we primarily found evidence for positive directional selection for both baseline and stress-induced corticosterone. For all measures of corticosterone, the vast majority of samples came from the low end of the observed range of values, which limits our ability to draw inferences about survival at higher trait values. Thus, we can say with some certainty that positive directional selection acts on the lower end of the range of baseline and stress-induced values, but we

Table 5 Linear (β) selection gradients measuring 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 response					
Age = 1	−0.016	0.053	0.085	1,13	0.775
Age > 1	0.032	0.041	0.608	1,25	0.443
Maximal free CORT response					
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 ($P < 0.05$).

cannot draw strong conclusions about the shape of the selection at mid-to-high trait values. In fact, glucocorticoids have potent and often deleterious effects when secreted at high levels over extended time frames (whether levels remain continuously elevated in response to chronic stress or are repeatedly elevated in response to multiple acute stressors; see Korte *et al.*, 2005 for review). So, we would predict that the more deleterious effects of elevated glucocorticoids (e.g. suppressed immune function, neural remodelling and subsequent degeneration, and excessive glucose mobilization with eventual insulin insensitivity, Sapolsky *et al.*, 1986) would have long-term longevity costs and therefore lead to a decline in survival. We did not see this in our analysis, but with fewer individuals at high CORT levels, we likely had reduced power to detect survival effects in that elevated range.

The data from other species are mixed. Positive directional selection for stress-induced corticosterone was found in rabbits (Cabezas *et al.*, 2007), and baseline corticosterone has been positively related to survival in two species of lizards (Comendant *et al.*, 2003; Cote *et al.*, 2006). However, storks showed negative directional selection on baseline corticosterone (Blas *et al.*, 2007). In another endocrine system, juncos showed evidence of stabilizing survival selection androgen response to challenge (McGlothlin *et al.*, 2010).

Finally, we found evidence suggesting that the shape of the relationship between some measures of stress-induced free glucocorticoids and survival varied across years. For the first 4 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 cannot confidently state that selection changes through time. Nevertheless, these data suggest that fluctuating selection may contribute to the maintenance of variation in endocrine traits in natural populations. Recent emphasis has been placed on the importance (Williams, 2008) and sources (Kempenaers *et al.* 2008) of individual variation in endocrine parameters, but it remains an open question. We hope this study encourages other endocrinologists validate our findings.

Fecundity selection

For reproductive success, we found contrasting directional selection in baseline (positive) and stress-induced fold-increase in corticosterone (negative). Whereas our data suggest a linear increase in reproduction with increasing baseline corticosterone, other studies suggest that reproduction would begin to suffer as corticosterone reaches stress-induced levels (Wingfield & Sapolsky, 2003). Previous studies of glucocorticoids and reproduction also found primarily directional selection (both positive and negative) between glucocorticoids and reproduction (e.g. tree swallows, *Tachycineta bicolor*: Bonier *et al.*, 2009b; Eastern fence lizard *Sceloporus undulatus*: John-Alder *et al.*, 2009; snow petrels, *Pagodroma nivea*: Goutte *et al.*, 2010).

The relationship between corticosterone and reproductive success was present only in females in our study. In this species, females build the nest, provide all of the incubation and perform the majority of offspring provisioning (Morton, 2002). Also, female reproductive success is more dependent on the success of their nest than males, because extra-pair paternity in this population is 30–56% (MacDougall-Shackleton *et al.*, 2002). Together, these facts may explain why female glucocorticoid profiles are related to number of offspring fledged

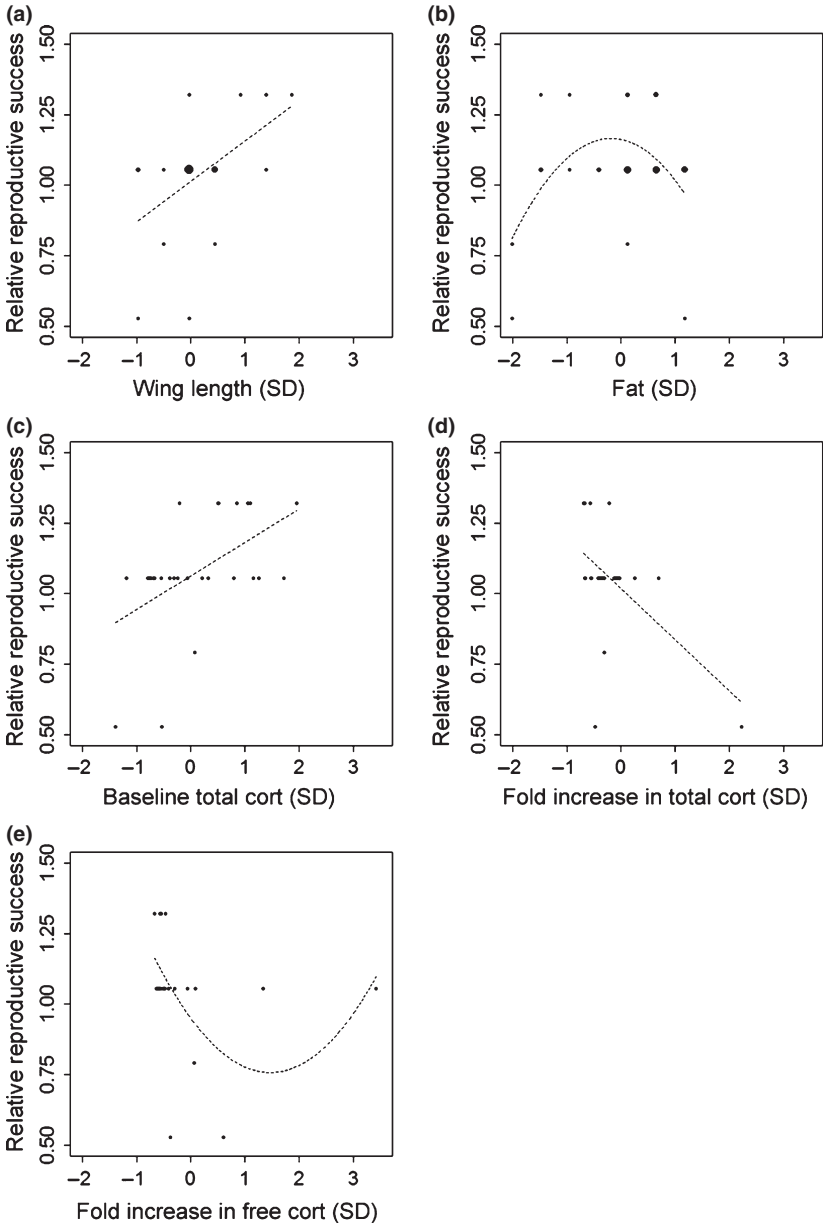


Fig. 5 Relationship in returning breeders between relative fitness (individual reproductive output/population mean reproductive output) and standardized individual covariates ((individual value − population mean value)/population standard deviation). Size of symbol represents number of points for morphological traits. All relationships are significant at $P < 0.05$. (a,b) Morphological/energetic covariates. (c) Baseline corticosterone covariates. (d,e) Stress-induced corticosterone covariates.

but males' glucocorticoid profiles are not. Males' fitness may depend more on their ability to obtain 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 to capture a relationship between corticosterone and reproductive success in males.

Trade-off between survival and reproduction

The two main components of fitness, survival and reproduction, are thought to trade off within an individual such that an increase in one is necessarily accompanied by a decrease in the other (Williams, 1966). The existence of this trade-off has been shown in a variety of systems (Reznick *et al.*, 1986), although it can be obscured by differences in resource acquisition within species (van Noordwijk & de Jong, 1986; Reznick *et al.*, 2000). Glucocorticoids have been put forward as a candidate mechanism mediating the trade-off between survival and reproduction (Ricklefs & Wikelski, 2002), because stress-induced glucocorticoids are thought to promote survival-oriented behaviour and physiology at the expense of other functions like reproduction (Wingfield *et al.*, 1998). Our results supported this: higher stress-induced glucocorticoids were associated with reduced reproduction and increased survival.

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

Trait	γ	SE	t	df	P
Wing length					
Age = 1	0.01	0.024	0.407	2,12	0.692
Age > 1	0.012	0.055	0.221	2,21	0.827
Mass					
Age = 1	0.031	0.03	1.053	2,12	0.313
Age > 1	−0.035	0.036	0.977	2,27	0.337
Body condition (SMI)					
Age = 1	−0.013	0.031	0.422	2,12	0.68
Age > 1	0.02	0.031	0.661	2,21	0.516
Fat					
Age = 1	−0.064	0.045	1.41	2,12	0.184
Age > 1	**−0.107**	**0.038**	**2.8**	**2,27**	**0.009**
Baseline total CORT					
Age = 1	0.007	0.032	0.205	2,12	0.841
Age > 1	−0.055	0.044	1.261	2,24	0.219
Baseline free CORT					
Age = 1	−0.013	0.041	0.318	2,12	0.756
Age > 1	0.056	0.074	0.758	2,21	0.457
Maximal total CORT response					
Age = 1	−0.001	0.034	0.027	2,12	0.979
Age > 1	−0.03	0.031	0.978	2,24	0.338
Max free CORT response					
Age = 1	0.152	0.194	0.784	2,12	0.448
Age > 1	0.055	0.042	1.303	2,21	0.207
Integrated total CORT					
Age = 1	−0.016	0.048	0.339	2,11	0.741
Age > 1	−0.03	0.046	0.642	2,20	0.528
Integrated free CORT					
Age = 1	0.029	0.083	0.353	2,11	0.731
Age > 1	0.051	0.046	1.106	2,19	0.283
Fold-increase in total CORT					
Age = 1	0.017	0.031	0.556	2,12	0.589
Age > 1	−0.04	0.066	0.606	2,20	0.551
Fold-increase in free CORT					
Age = 1	0.017	0.069	0.252	2,12	0.805
Age > 1	**0.089**	**0.039**	**2.28**	**2,19**	**0.034**

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

This is the first time both sides of this trade-off with direct measures of survival and reproduction have been related to glucocorticoid secretion within a single population. An experimental study looking at indirect measures of survival and reproduction found a similar pattern. A recent study by Almasi *et al.* (2013) found that an experimental elevation of glucocorticoids reduced home range size and activity in male barn owls (*Tyto alba*) and reduced body mass loss in females (results were dependent on feather colouration); they conclude that these represent a decline in reproductive behaviour to favour survival. However, they also found that glucocorticoid implant increased brooding behaviours in females (again dependent on feather colouration), which supports a positive relationship between elevated glucocorticoids and reproduction. The strongest 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.

Relationship between glucocorticoids and fitness

There are three primary hypotheses regarding the relationships between corticosterone and fitness. The CORT-trade-off hypothesis (described above) posits that elevated glucocorticoids increase energy expended on survival at a cost to reproduction. This hypothesis can be applied to 'baseline' or 'stress-induced' CORT levels, which estimate the normal allostatic state of the animal and the ability of the adrenal to respond to stress, respectively. Alternatively, the CORT-fitness and the CORT-adaptation hypotheses deal only with baseline corticosterone levels (Bonier *et al.*, 2009a). The former suggests that elevated glucocorticoids will always be associated with a decline in fitness (reproduction and survival), whereas the latter suggests that elevated glucocorticoids may benefit reproductive effort (via increasing foraging for young or energy spent on parental care). Our results run contrary to the CORT-fitness hypothesis as both survival and reproductive success were positively associated with our measures of baseline corticosterone. As such, our data provide support for both the CORT-adaptation hypothesis (elevated baseline is beneficial for reproduction and survival) and the CORT-trade-off hypothesis (elevated levels of stress-induced CORT favour survival over reproduction).

In correlational studies such as this one, it is always difficult to assess causality. Of particular concern is the issue of indirect selection. Correlations between traits complicate selection analyses, because selection on one trait can cause changes in the distribution of the correlated trait. This can result in the appearance of selection on the correlated trait (Lande & Arnold, 1983). In our study, it is difficult to separate out corticosterone from individual quality. Baseline corticosterone during the arrival phase appears to reflect individual quality because both survival and reproductive output covary with baseline corticosterone (Figs 2 and 5c). Our data do not address the causality of this relationship. Individual quality could drive 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 mates during the arrival phase. Corticosterone increases in response to activity levels and energy expenditure (reviewed in Landys *et al.* 2006), which could lead to the observed positive association. Conversely, higher levels of glucocorticoids may drive the 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.

Another possible explanation for the apparent relationship between corticosterone and survival are differences in personality. Corticosterone profiles are known to be associated with personality and it is possible that our recapture rate is driven by differences in personality rather than survival. While possible, this scenario has several arguments against it. The proactive-reactive personality continuum frames the relationship between personality traits and corticosterone profiles. Birds with proactive personalities are characterized by more active behavioural responses and less-pronounced corticosterone stress responses as compared to reactive birds (Cockrem, 2007). If personality were driving the patterns in our data, we would expect a negative association between stress-induced corticosterone and survival; proactive individuals would be more likely to enter a trap, show greater survival and have lower stress-induced corticosterone. However, our data show a positive relationship between stress-induced corticosterone and survival (Fig. 3). Furthermore, the trapping effort is quite thorough and we believe that most resident individuals are captured each year. Thus, we do not believe that difference in personality is the primary driver of our survival estimates.

Alternatively, the positive relationship between endogenous corticosterone and fitness may simply reflect an age bias in adult arrival times. Previous work on this population found that older birds (returning breeders) tend to arrive earlier than first year breeders (Morton, 2002) and, in this study, older birds had greater reproductive output than younger birds. Further, corticosterone is often up-regulated during the breeding season (Romero & Wingfield, 1999), which could cause the early-arriving older birds to exhibit higher corticosterone levels. Although this set of circumstances could drive a relationship between corticosterone and reproductive success, we found no relationships between age and sample date, age and corticosterone, sample date and corticosterone, or sample date and reproductive success. We therefore reject this alternative hypothesis that positive relationship between corticosterone and reproductive success is an artefact of age or sampling date.

Patterns of selection

Directional selection on physiological traits was similar in magnitude to selection on wing length, the one morphological trait that demonstrated directional selection (Table 5). The relative strength of selection on physiological vs. morphological traits is a poorly documented comparison, so this represents an important data point for our understanding of how natural selection operates on physiological traits as compared to how selection acts on morphological traits. In another model, species for studying selection on physiological traits in natural populations, McGlothlin *et al.* also found evidence of stronger selection operating on an endocrine trait (McGlothlin *et al.*, 2010) than on morphological traits (McGlothlin *et al.*, 2005). However, the selection gradient varied depending on the trait and fitness component in question.

Fat score and fold-increase in free corticosterone both had significant quadratic correlations with of reproductive success (Table 6). The relationship between fat score and fecundity success was convex, suggesting stabilizing selection. Curiously, the relationship between reproductive success and fold-increase in free CORT had a concave shape, which suggests disruptive selection. However, given the relatively small number of data points ($n = 22$), we are reluctant to place too much weight on these findings.

It is important to be mindful of limitations on our ability to make inferences based on these data. Kingsolver *et al.* (2001) cautioned those undertaking selection studies about lack of replication and small sample sizes. Our study spans multiple years, but it focuses on a single population and was not replicated. Given the effort required to produce the current study, replication is not practical. Unreplicated studies should always be interpreted cautiously and we encourage others to undertake similar studies in other populations. Small sample sizes lower statistical power and increase standard error. Our sample sizes varied by analysis: the Mark 'recaptures only' survival analysis had *n* approximately 200 for both baseline and stress-induced per analysis; the reproductive selection gradient analysis was much more limited, with between 36 and 45 individuals per analysis.

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) vs. total hormone concentrations (Breuner & Orchinik, 2002; Romero, 2002). Various studies have found support for importance of total hormone concentrations (e.g. European wild rabbits: Cabezas *et al.*, 2007; and tree swallows: Bonier *et al.*, 2009b), whereas some support the importance of free hormone concentrations (e.g. white-crowned sparrows: Breuner *et al.*, 2003; European starlings: Love *et al.*, 2004; and barn owls: Almasi *et al.*, 2009). Hence, this remains an open question in the literature. Fitness components are integrated measures of biological performance, so we examine our results in light of this debate. For survival probability, models with stress-induced free corticosterone were better supported than models with stress-induced total corticosterone. This suggests that free hormone levels may be more relevant for survival; however, the level of support was similar between models including total and free baseline corti-

costerone. Conversely, for fecundity, only measures of total corticosterone showed significant linear relationships, suggesting that total hormone levels may be more relevant for reproduction. However, fold-increase in free corticosterone had a significant quadratic relationship with reproductive output. Overall, our results do not exclusively support either side of the total vs. free hormone debate.

Endocrinologists further disagree on which measure of stress-induced glucocorticoids best reflects the glucocorticoid response to stress, and resolving this debate would refine our understanding of glucocorticoid's mechanism of action. Romero (2004) argues that the integrated response (total hormone secreted over sample period) is the most relevant measure of the glucocorticoid stress response, whereas Breuner (2010) argues in favour of the relevance of the fold-increase in corticosterone. For survival probability, we found that models with maximal response and integrated (free) corticosterone fit better than models with fold-increase. However, the opposite pattern was found in reproduction, where fold-increase in total and free corticosterone was the only factor significantly correlated with reproduction. If baseline and stress-induced measures are correlated, one can account for variation in baseline CORT as part of the stress-induced model. This is an elegant solution, but we did not use it here given that our baseline and stress-induced levels were not highly correlated (adjusted $R^2 = 0.14$).

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 fecundity 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.

Acknowledgments

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