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The relationship between fitness and baseline glucocorticoids in a passerine bird

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ABSTRACT

Glucocorticoid (cort) hormones are increasingly applied in studies of free-ranging animals, with elevated baseline cort levels generally assumed to indicate individuals or populations in worse condition and with lower fitness (the *Cort-Fitness Hypothesis*). The relationship between cort and fitness is rarely validated and studies investigating the cort-fitness relationship often find results inconsistent with the Cort-Fitness Hypothesis. The inconsistency of these studies may result in part from variation in the cort-fitness relationship across life history stages. Here we address the following questions in a two-year study in free-ranging tree swallows (*Tachycineta bicolor*): (1) Do baseline cort levels correlate with fitness within a life history stage? (2) Does the cort-fitness relationship vary across different life history stages? (3) Does the cort-fitness relationship vary across life history stages within an individual? (4) Does reproductive effort influence cort levels, and do cort levels influence reproductive effort? We measured baseline cort and fitness components in female birds of known breeding stages. We find correlations between baseline cort levels and fitness within some life history stages, but the relationship shifts from negative during early breeding to positive during late breeding, even within the same individuals. A positive relationship between baseline cort and fitness components during the nestling period suggests that reproductive investment may elicit higher cort levels that feedback to reallocate more effort to reproduction during critical periods of nestling provisioning. Our findings provide reason to question the Cort-Fitness Hypothesis, and have implications for the application of cort measures in monitoring the condition of populations of conservation concern.

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1. Introduction

Ever since the advent of nonlethal field endocrine techniques (Wingfield and Farner, 1976), biologists have sought to interpret the significance of variation in hormone levels in free-ranging animals. In recent years, measures of glucocorticoid (*cort* or "stress" hormone) levels have been increasingly employed as physiological indices of individual and population condition or health (reviewed in Walker et al., 2005a; Wikelski and Cooke, 2006). In general, elevated baseline cort levels are assumed to indicate individuals or populations in worse condition and with lower fitness (hereafter termed the *Cort-Fitness Hypothesis*) (e.g., Arlettaz et al., 2007; Creel et al., 2002; Marra and Holberton, 1998; Sands and Creel, 2004; Thiel et al., 2008; Walker et al., 2005b; Wasser et al., 1997).

The Cort-Fitness Hypothesis follows, in part, from cort's involvement in an organism's integrated physiological response to environmental and social challenges (Sapolsky et al., 2000). As challenges increase, cort levels increase, and fitness (i.e., lifetime reproductive success) is predicted to decline simply because limited resources must be reallocated from normal activities of survival and reproduction to responding to challenges (Waelti and Reyer, 2007; Zera and Harshman, 2001). Thus high cort levels may signify individuals encountering difficult challenges that reduce their relative fitness. Further support for the hypothesis comes from frequently-found negative correlations between variation in baseline cort levels and indices of body condition and habitat quality (e.g., Jenni-Eiermann et al., 2008; Johnson, 2007; Muller et al., 2007; Oppliger et al., 1998; Waye and Mason, 2008; Williams et al., 2008).

The correlation between baseline cort levels and fitness is generally not considered to be directly causal. Instead, cort levels are thought to covary with other factors that directly influence fitness (e.g., resource availability, fat stores, habitat quality), in addition to cort's direct role in mediating allocational trade-offs (Bonier et al., 2007; French et al., 2007; Love et al., 2005; Owen-Ashley et al., 2004). A direct reduction of fitness caused by chronically elevated cort associated with chronic stress has been observed but is

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thought to occur only with long-term elevation of cort above normal baseline levels (Moore et al., 2005; Sapolsky, 1985; Young et al., 2006). Within baseline levels, cort does not interfere with reproductive effort, and in some instances cort can actually enhance reproductive success (Michael et al., 2003; Moore and Jessop, 2003; Wingfield and Sapolsky, 2003). Furthermore, increases in cort levels may actually facilitate reproductive effort (and, by extension, increase fitness) during peak periods of energetic demand during reproduction (Love et al., 2004; Romero, 2002). Increased cort levels in response to experimentally increased reproductive effort have been documented (e.g., through brood size manipulation, Ilmonen et al., 2003; Saino et al., 2003), but the implications of these findings for the Cort-Fitness Hypothesis have not been discussed.

Despite the pervasiveness of the Cort-Fitness Hypothesis in the literature, the relationship between cort and fitness is rarely validated. Studies investigating the cort-fitness relationship often find results inconsistent with the Cort-Fitness Hypothesis, including no relationship (e.g., Angelier et al., 2007a,b; Blas et al., 2007), non-linear correlations (Brown et al., 2005), and positive correlations (e.g., Beletsky et al., 1992; Cyr and Romero, 2007; Silverin, 1998), as well as the predicted negative correlation between cort and fitness (e.g., Angelier et al., 2007a,b; Clinchy et al., 2004; Romero and Wikelski, 2001). Relationships sometimes vary within studies, depending on sex of the individual (Wingfield et al., 1999), timing of sampling (Kitaysky et al., 2007), fitness metric used (Angelier et al., 2007b), and reproductive strategy of the individual (Lancaster et al., 2008). The inconsistency of these findings with the Cort-Fitness Hypothesis may result in part from variation in the cort-fitness relationship across life history stages and contexts, where selection may favor low cort in some stages and contexts and high cort in others (Romero, 2002). Variation in cort levels across life history stages is well described (Romero, 2002), but the potential for variation in the cort-fitness relationship across life history stages has not been well studied.

Here we address the following questions: (1) Do baseline cort levels correlate with fitness within a life history stage? (2) Does the cort-fitness relationship vary across different life history stages? (3) Does the cort-fitness relationship vary across life history stages within an individual? (4) Does reproductive effort influence cort levels, and do cort levels influence reproductive effort? We find correlations between baseline cort levels and fitness within some life history stages, but the relationship shifts from negative during early breeding to positive during late breeding, even within the same individuals. A positive relationship between baseline cort and fitness components during the nestling period suggests that initial reproductive investment may elicit higher cort levels that feedback to reallocate more effort to reproduction during critical periods of nestling provisioning.

2. Materials and methods

2.1. Study system

All methods described herein conform to the regulatory standards of the Queen's University Animal Care and Use Committee. We conducted this study in May and June of 2007 and 2008 in a free-ranging population of box-nesting tree swallows (*Tachycineta bicolor*, Fig. 1) at the Queen's University Biological Station near Chaffey's Lock, Ontario, Canada (44°34′ N, 76°19′ W, ~135 m elevation). *T. bicolor* is a migratory swallow found breeding throughout much of North America (Robertson et al., 1992). Box-nesting species offer a unique opportunity for repeated sampling of individuals of known breeding stage, and thus are ideal for this study. The Queen's University population of *T. bicolor* has been the subject of

Fig. 1. Picture of the study species, *Tachycineta bicolor*, and an example of the nest boxes that the focal population at the Queen's University Biological Station (Chaffeys Lock, Ontario, Canada) uses for breeding. Photo courtesy of P–G Bentz.

intense monitoring and study for more than three decades (cf. Dunn et al., 1994; Robertson and Rendell, 2001; Stapleton et al., 2007; Whittingham et al., 1992). All breeding individuals and nestlings in the study population are marked with numbered leg bands for individual identification. *T. bicolor* have high breeding site fidelity, with more than 85% of individuals in a nearby (New York, USA) box-nesting population returning to their initial breeding site (Winkler et al., 2004). The entire study site consists of eight grids of 6–35 nest boxes placed in hay fields and 24 solitary boxes distributed along a road connecting the study grids. For the present study, we focused on birds nesting on one grid of 35 boxes in 2007, and this grid plus two additional grids of 23 and 25 boxes in 2008. Cort levels did not differ among sampling grids. The 35- and 23-box grids are set up with identical inter-box spacing of 40 m along each row and 28 m diagonally among rows. The 25-box grid is set up with two rings of boxes, one evenly spaced around the perimeter of a swamp in the center of the field and one around the outer perimeter of the hay field.

2.2. Sample collection and field monitoring

We only include females in the present study. To control for diel variation in cort levels, we trapped birds between 9 and 11:45 a.m. inside their nest boxes either with trap-doors set on the entryways (set when the bird was out of the box and closing upon the bird's return) or by placing our hands over the entryway when the bird was inside the box. Method of capture did not influence measured cort levels (Student's *t*-test, d.f. = 55, *t* = 0.224, *P* = 0.824). Once captured, we collected a small (<150 μL) sample of blood into heparinized microcapillary tubes through puncture of the brachial vein. Blood was sampled within 3 min of the time when females were trapped in the boxes. After blood sampling, we collected morphometric measures from all birds and placed leg bands on any unbanded birds. We measured mass (to nearest 0.1 g with a Pesola spring scale), tarsus length (to nearest 0.1 mm with a caliper), and wing length (to nearest 0.1 mm with a wing ruler) of each bird. We then released the bird, usually within 10 min of capture. We stored all blood samples on ice until they could be transported to the lab for processing.

We recorded breeding activity of all focal birds through routine monitoring of nest box contents. We checked nest boxes once every three days until the appearance of the first egg in the nest box. We determined onset of incubation by monitoring egg-laying behavior daily – *T. bicolor* lay one egg every day, with an occasional skipped day, until clutch completion and then initiate incubation

behavior (Robertson et al., 1992). Once incubation was initiated, we again monitored box contents every three days until all offspring fledged or the breeding effort failed.

During 2007, we captured 15 birds on day 3 or 4 after onset of incubation. We then monitored the outcome of those 15 nesting attempts and recorded the number of offspring that reached the age of departure from the nest box (*fledging success*). During 2008, we captured 14 birds 4–12 days prior to initiation of incubation (*nest-building*), 16 birds on day 2 or 3 of the incubation period (*early incubation*), 20 birds on day 7–9 of the incubation period (*late incubation*), and 16 birds on days 3–5 and 8 birds on days 10–12 of the period of nestling provisioning (*nestling*). Because of high rates of nest predation in 2008, we pooled samples collected early and late in the nestling period. Results do not differ if these data are considered separately. In total, we sampled 39 adult females during 2008, with 13 females sampled once, 17 sampled twice, and 9 sampled three times. No females were sampled more than once during a given breeding stage. In 2008 we recorded fledging success as described above and also collected measures of clutch mass and nestling growth rate. We measured clutch mass on day 2 of the incubation stage and nestling mass (to nearest 0.1 g using an electric balance) on days 4 and 12 of the nestling period to assess nestling growth (analyzed as change in mass of the entire brood over an 8-day period).

2.3. Hormone assay

We centrifuged blood samples within ten hours of collection to separate plasma, which was then stored at $-20\,^{\circ}C$ until assay. We quantified plasma levels of total corticosterone (the primary glucocorticoid in birds) in each sample in duplicate through direct radioimmunoassay, following extraction with re-distilled dichloromethane (see Wingfield et al., 1992 for details). All samples from each year were assayed simultaneously (2 assays total). Within-assay variation among replicate known-concentration standard samples was 5.8% (2 standards) and 10.4% (5 standards) in 2007 and 2008, respectively. Inter-assay variation was 14.7%. Hormone data from the two assays were analyzed separately.

2.4. Estimates of fitness components

We used four measures of fitness components and reproductive effort in our analyses. For our 2007 data, we used fledging success (as defined above) as an estimate of reproductive success and return rate in 2008 for birds sampled in 2007 as an estimate of mortality or dispersal. In 2008, we used clutch mass as a measure of a fitness component. Clutch mass reflects variance in fecundity (number and size of eggs laid), which contributes directly to fitness (Monaghan and Nager, 1997; Williams, 1994). We recorded fledging success in 2008, but do not use it as a fitness measure because of high rates of predation. All of the nest boxes on the study site are equipped with predator guards designed to deter predation. In 2008, a small number of large *Elaphe obsoleta* (black rat snake) evaded our predator guards and depredated a majority of the nest boxes (32 of 46 occupied nest boxes on three focal grids). Thus variance in fledging success in 2008 was largely driven by effectiveness of the predator guards, rather than any biologically relevant factor. In 2007, none of the 15 focal nests were depredated, and variance in fledging success was largely driven by variation in clutch size (ranging from 4 to 7 eggs) and partial brood survival through inclement weather (nine nests had partial brood mortality, three suffered complete brood mortality). Finally, we use change in brood mass over an 8-day period in 2008 as a measure of reproductive investment. Change in brood mass captures variation in number of offspring

and in growth rate, and thus reflects variation in fecundity, hatching success, and/or nestling provisioning.

2.5. Statistical analyzes

We log-transformed all cort levels and squared all clutch masses to meet assumptions of normality. We conducted all statistical analyses using the programs R (Crawley, 2005) and JMP. We addressed our first question (do baseline cort levels correlate with fitness within a life history stage?) using a generalized linear model (GLM) with a Poisson distribution and a log link function, with number of offspring fledged in 2007 as the response variable and maternal cort levels measured on day 3–4 of the incubation stage as an independent factor. We also tested if cort levels and fledging success differed between birds that returned the following year ($N = 10$) and those that did not ($N = 5$), using a Wilcoxon Rank Sums test.

We addressed the second question (does the cort-fitness relationship vary across life history stages?) using a GLM with a normal distribution and identity link function, with clutch mass measured in 2008 as the response variable, and breeding stage, maternal cort levels, and an interaction term as independent factors. A significant effect of the interaction term would indicate that the relationship between cort levels and clutch mass varied with breeding stage.

We addressed the third question (Does the cort-fitness relationship vary across life history stages within an individual?) using data collected from 10 females sampled both during the early incubation and nestling stages. We used a GLM with a normal distribution and identity link function, with percent change in log maternal cort levels within an individual from the early incubation stage to the nestling stage as the response variable and clutch mass as an independent factor. If reproductive effort directly or indirectly influences cort levels, we predicted that clutch mass (determined prior to onset of incubation) should predict the subsequent change in cort through the nestling period. We addressed this final question (does reproductive effort influence cort levels, and do cort levels influence reproductive effort?) using a GLM with a normal distribution and identity link function, with maternal cort levels measured during the nestling stage as a response variable and change in brood mass over a period of 8 days as an independent factor. A significant relationship would suggest that investment in offspring provisioning directly or indirectly interacts with cort levels.

3. Results

Do baseline cort levels correlate with fitness within a life history stage? Females with lower cort during early incubation fledged more offspring (Fig. 2, $N = 15$, d.f. = 14, $Z = -2.847$, $P = 0.004$; 2007 data). Females that returned to the study site in 2008 fledged more offspring (Fig. 3, d.f. = 1, $X^2 = 9.832$, $P = 0.002$) and had lower cort levels during early incubation (Fig. 3, d.f. = 1, $X^2 = 4.335$, $P = 0.037$) in 2007 than females that did not return.

Does the cort-fitness relationship vary across life history stages? The cort-fitness relationship varied across life history stage (effect of the interaction between cort level and life history stage, $N = 74$, d.f. = 3, $F = 4.533$, $P = 0.006$; 2008 data). Cort negatively correlated with clutch mass during early incubation (Fig. 4a, $N = 16$, $\beta = -53.297$, $P = 0.009$), but positively correlated with clutch mass during the nestling stage (Fig. 4b, $N = 24$, $\beta = 36.436$, $P = 0.031$). Cort did not covary with clutch mass during other stages (nest-building, $N = 14$, $\beta = -28.98$, $P = 0.23$, late incubation, $N = 20$, $\beta = -20.95$, $P = 0.32$).

Does the cort-fitness relationship vary across life history stages within an individual? Females with heavier clutches of eggs had greater increases in baseline cort from early incubation to the nest-
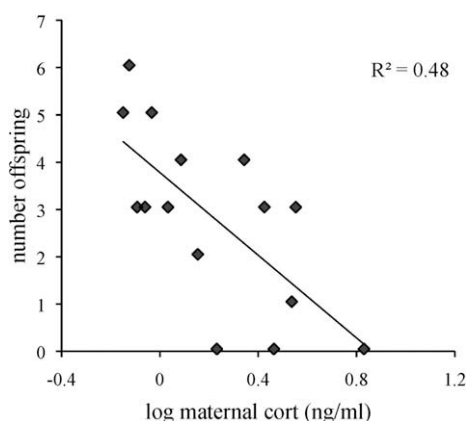
Fig. 2. Number of fledged offspring plotted against log of maternal cort measured early in the incubation stage of *Tachycineta bicolor*. Female birds with higher cort levels fledged fewer offspring (GLM with Poisson distribution, $N = 15$, d.f. = 14, $Z = -2.847$, $P = 0.004$).
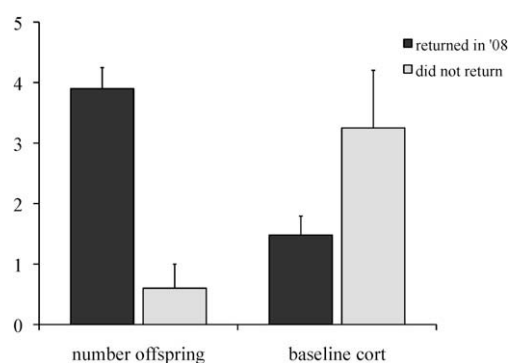

Fig. 3. Number of fledged offspring and baseline cort levels (ng/ml) (mean + SE) for *Tachycineta bicolor* from the 2007 study that did ($N = 10$) and did not ($N = 5$) return to the study site in 2008. Females that returned to the study site in 2008 fledged more offspring (Wilcoxon Rank Sums test, d.f. = 1, $X^2 = 9.832$, $P = 0.002$) and had lower cort levels during early incubation (Wilcoxon Rank Sums test, d.f. = 1, $X^2 = 4.335$, $P = 0.037$) in 2007 than females that did not return.

ling stage (Fig. 5, $N = 10$, d.f. = 1,8, $F = 11.134$, $P = 0.010$). This relationship found within individuals parallels the pattern observed among individuals (Fig. 4).

Does reproductive effort influence cort levels, and do cort levels influence reproductive effort? Females with heavier clutch masses had greater increases in baseline cort across breeding stages (Fig. 5, above). In addition, females with high cort during the nestling provisioning period had broods that increased more in mass over an 8-day period (Fig. 6, $N = 8$, d.f. = 1,6, $F = 7.348$, $P = 0.035$).

4. Discussion

The relationship between baseline cort and fitness varied across life history stages in our population of *T. bicolor*. Early in incubation, maternal cort negatively covaried with both reproductive success (Figs. 2 and 4a) and the likelihood that females returned to breed on our sites the following year (Fig. 3). This negative relationship between cort and fitness, evident early in incubation, completely reversed later in breeding – cort positively covaried with fitness during nestling provisioning (Fig. 4b). This reversal of the cort-fitness relationship was even evident within the same individuals: birds that invested more in reproduction (as reflected in heavier clutch masses) had greater increases in baseline cort levels from early incubation through to the nestling period (Fig. 5). In addition, females with higher baseline cort during nestling provi-
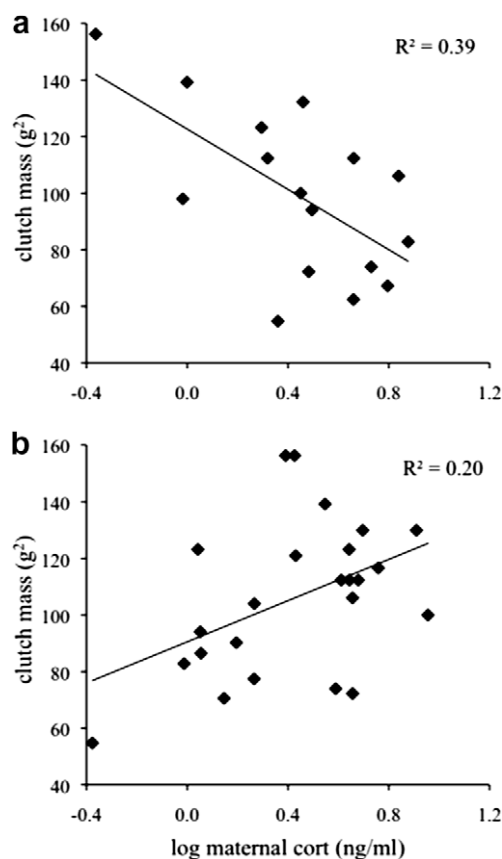

Fig. 4. Clutch mass (g^2) of female *Tachycineta bicolor* plotted against the log of maternal cort measured early in the incubation stage (A) and during the nestling stage (B). Females with higher clutch masses had lower cort levels early in breeding (A, GLM, $N = 16$, $\beta = -53.297$, $P = 0.009$), while females with higher clutch masses had higher cort levels late in breeding (B, GLM, $N = 24$, $\beta = 36.436$, $P = 0.031$).
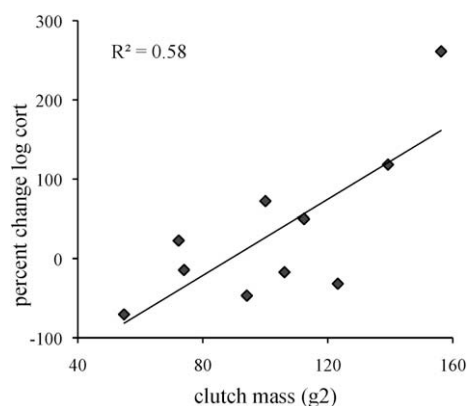

Fig. 5. Percent change in the log of maternal cort between the early incubation and nestling stages of *Tachycineta bicolor* plotted against clutch mass (g^2). Individual females with higher clutch masses had greater increases in cort levels through their breeding attempt (GLM, $N = 10$, d.f. = 1,8, $F = 11.134$, $P = 0.010$).

sioning had broods that increased more in mass (Fig. 6). If investment in offspring growth promotes offspring survival, then females with higher baseline cort late in breeding would be predicted to have higher fitness. In the present study, we focus only on female *T. bicolor*, and we do not know whether similar changes in cort-fitness relationships will be evident in male animals, particularly in cases when males show reduced investment in reproduction.

Variation in the relationship between cort and fitness across life history stages may explain the lack of consistent patterns between
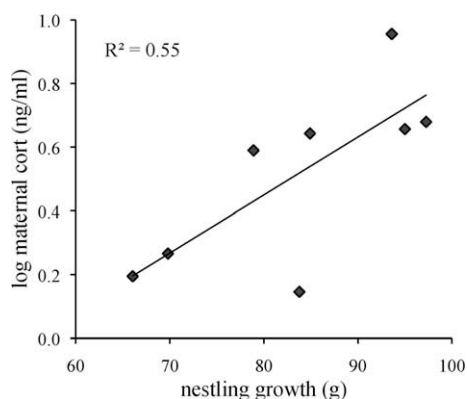
Fig. 6. Log of maternal cort measured during the nestling stage of *Tachycineta bicolor* plotted against nestling growth (change in mass of entire brood in grams over an 8-day period). Individual females with greater increases in the mass of their broods had higher cort during the period of nestling provisioning (GLM, $N = 8$, d.f. = 1,6, $F = 7.348$, $P = 0.035$).

cort and fitness in some previous studies that did not control for breeding stage (e.g., Brown et al., 2005). Use of a box-nesting population of birds in the present study facilitated repeated sampling of individuals of known breeding stage. Had we pooled our data across all breeding stages, we would have found no relationship between baseline cort levels and fitness. The implications of these findings for the use of cort in conservation are dramatic. Baseline cort levels measured during certain breeding stages may be considerably more useful for assessing population and individual health than those measured at other stages. Furthermore, the conclusions drawn from interpretation of baseline cort levels may vary depending on timing of sampling, with high baseline cort interpreted as negative at certain life history stages, and positive at others.

Female birds with heavier clutches had lower cort early in breeding and higher cort later in breeding (Figs. 4 and 5). While initially this pattern may seem counterintuitive, it may simply reflect the role of cort in an adaptive reproductive strategy. A female arriving on the breeding grounds in good condition may invest heavily in reproduction, laying more eggs or larger eggs. That female would be expected to have low baseline cort because of the negative relationship between cort and individual condition (Oppliger et al., 1998; Waye and Mason, 2008). Later in the same breeding effort, cort levels may rise to mediate the challenges of provisioning a large brood, with cort promoting the reallocation of resources to address high reproductive demands (potentially even at the expense of the female's own condition). Thus we find a positive relationship between cort and reproductive effort later in breeding (Figs. 4b, 5, and 6) that may reflect the role of cort in an adaptive strategy to reallocate energy to reproduction. Studies showing increases in cort in response to experimentally increased brood sizes (Saino et al., 2003) support the idea that reproductive demand can positively influence cort levels. Because of the iterative nature of the interaction between cort, the environment, individual condition, and behavior, we might also expect a positive feedback loop, with high reproductive effort and investment driving increases in cort levels, which themselves may cause increased reproductive investment (e.g., through increased nestling provisioning).

Our results provide reason to question the Cort-Fitness Hypothesis, wherein high cort reflects individuals or populations with low relative fitness. If our findings can be generalized across other taxa, then the Cort-Fitness Hypothesis must be revised. We propose that this revised hypothesis be termed the Cort-Adaptation Hypothesis. At the time when an individual makes "decisions" regarding reproductive investment, we might expect to find the commonly predicted negative relationship between baseline cort and fitness because individuals in good condition are able to invest more in reproduction. After an individual in good condition invests heavily in reproduction, we predict that baseline cort levels will increase over time to positively correlate with fitness because increased investment in reproduction requires increases in cort levels that promote reallocation of energy to reproduction. This revision of the Cort-Fitness Hypothesis is not in contradiction to the foundations of the original hypothesis, which relies on the assumption that baseline cort levels increase with increasing social or environmental challenges that tend to negatively covary with fitness. Instead, we expand our interpretation of challenges to include those challenges associated with reproduction. The challenge of increasing reproductive investment will positively covary with fitness and baseline cort levels, leading to our expectation under the Cort-Adaptation Hypothesis of a positive cort-fitness relationship.

Measures of baseline and stress-induced cort are increasingly employed as tools for monitoring populations of conservation concern. Our findings should serve as a caution, underscoring the importance of carefully controlling for life history stage and validating the cort-fitness relationship prior to drawing conclusions regarding the relative health and fitness of populations.

Acknowledgments

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