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Ainsley Anne Furlonger

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**The relationship between HPA regulation and fitness in a free-living population of
song sparrows (*Melospiza melodia*)**

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Ainsley A. Furlonger

Graduate Program in Biology

**A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science**

**The School of Graduate and Postdoctoral Studies
University of Western Ontario
London, Ontario, Canada**

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THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

CERTIFICATE OF EXAMINATION

Supervisor

Dr. Elizabeth MacDougall-Shackleton

Co-Supervisor

Dr. Scott MacDougall-Shackleton

Supervisory Committee

Dr. Liana Zanette

Examiners

Dr. Jeremy McNeil

Dr. Yolanda Morbey

Dr. Elizabeth Hampson

Dr. Greg Thorn (Chair)

The thesis by

Ainsley Anne Furlonger

entitled:

**The relationship between HPA regulation and fitness in a free-living
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Date _____

Chair of the Thesis Examination Board

ABSTRACT

In a changing environment, individuals must have the ability to physiologically cope with unpredictable stressors. The endocrine response to stressors is one mechanism vertebrates can use. The corticosterone (CORT)-fitness hypothesis predicts a negative relationship between acute stress induced levels of CORT and fitness. I examined the effectiveness of hypothalamo-pituitary-adrenal (HPA) axis regulation through exogenous hormone stimulation and plasma CORT levels as a predictor for various fitness measures in free-living song sparrows (*Melospiza melodia*). CORT levels at 30 minutes and in response to dexamethasone injection were predictive of lay date in this population. However, no relationships were observed between HPA regulation and other measures of fitness, including clutch size, proportion of eggs hatched, and adult survival (return rate). Thus, although there was no support for the narrowly defined CORT-fitness hypothesis, this study in combination with prior work suggests that the relationship between HPA-regulation and fitness can vary with life-history stage and between years.

Keywords: Stress, CORT-Fitness Hypothesis, Song Sparrow, HPA Regulation, Stress Response

CO-AUTHORSHIP

Publications derived from this research will be co-authored by Kimberly L. Schmidt, for her work on data collection and hormone analysis. Scott MacDougall-Shackleton and Elizabeth MacDougall-Shackleton for providing funding and helping with data collection, writing, and conceptual assistance.

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Chapter 1:

INTRODUCTION

When unpredictable changes in the environment threaten an animal's homeostasis, it is important that it have the capacity, through rapid physiological and behavioural changes, to divert resources and energy to immediate survival (Wingfield 2003). The initiation of these responses may occur in response to unpredictable weather conditions, low food abundance, dominance interactions, changes in social status, human activity or threat of predation (Palme et al. 2005, Angelier et al. 2009, Shini et al. 2009). Because almost no habitats are completely static and predictable, understanding how animals deal with stressors is an important aspect of ecology and conservation biology. In vertebrates, the primary mechanism for dealing with these stressors is a highly conserved generalized stress response seen in all vertebrate species.

The vertebrate stress response is comprised of two main components: a sympathetic nervous system component, the fight or flight response, and a hormone-mediated (glucocorticoid) component, which occurs via the hypothalamic-pituitary-adrenal (HPA) axis. The neural response occurs nearly instantaneously, and involves up-regulation of the sympathetic autonomic nervous system and rapid release of epinephrine from the adrenal medulla. In comparison, the endocrine system functions more slowly, with physiological effects becoming apparent approximately 3 minutes after encountering the initial stressor. This endocrine response works to inhibit functions not essential for immediate survival such as reproduction and digestion, and stimulates mobilization of energy necessary to cope with the stressor.

The HPA-mediated endocrine response to stress occurs when neural signals reach the hypothalamus and trigger a release of corticotropin-releasing hormone (CRH). This initiates the release of adrenocorticotrophic hormone (ACTH) from the

anterior pituitary, which binds to receptors in the cortex of the adrenal gland and stimulates synthesis and release of glucocorticoids (primarily corticosterone [CORT] in birds) from the adrenal cortex (Wingfield & Sapolsky 2003). Elevated glucocorticoids have many effects, including mobilizing energy and inhibiting physiological systems not required for immediate survival. The stress response ends when glucocorticoids in the blood initiate negative feedback by binding to receptors in the brain and pituitary to inhibit further release of CRH and ACTH (Romero & Reed 2008). This physiological response to stress appears to be conserved across the majority of vertebrates studied (Wingfield et al. 1997, Bonier et al. 2009a).

In the short term, this stress response is presumably adaptive as it increases the ability to respond immediately with extreme physical exertion by mobilizing and producing glucose at the expense of fats and proteins (Wingfield et al. 1997). For short term or 'acute' stressors this physiological change is transient: once an individual is removed from the stressor, it can resume regular physiological functioning through negative feedback mechanisms. However, some stressors persist for an extended period of time (days or even longer). Such 'chronic' stressors may prevent the individual from returning to baseline conditions, which can be detrimental to health and survival (Wingfield et al. 1998, Breuner et al. 2008, Shini et al. 2009). For example, shifting blood flow and energy away from the digestive system is beneficial in the short term, but in the long term reduces digestive efficiency and leaves the gastro-intestinal tract susceptible to infection. Recent empirical and theoretical work suggest that an optimal response to stress is one where the individual has low baseline CORT concentrations, that can be rapidly increased in response to a stressor but also quickly return to baseline levels once the stressor has dissipated (Figure 1.1; Breuner et al. 2008, MacDougall-Shackleton et al. 2009). It is important to note that the optimal stress response is context dependent,

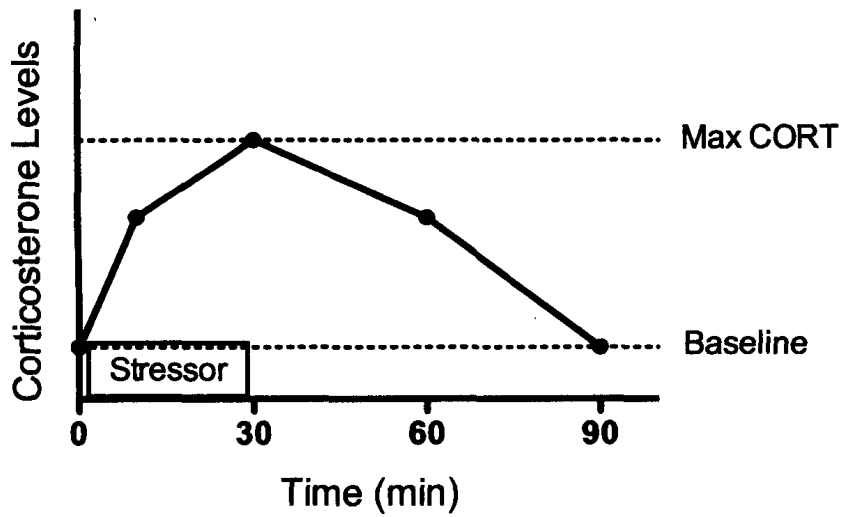


Figure 1.1 Schematic stress-response curve. Individual encounters stressor at time 0 and CORT levels in blood rise until approximate max CORT at 30 minutes. Following removal of stressors, individuals return to baseline CORT levels.

and can vary with time, place, and type of stressor. Quickly returning CORT to baseline levels enables the individual to avoid the costs associated with exposure to chronic stress, emphasizing the importance of negative feedback mechanisms in the stress response system.

The CORT-fitness hypothesis arises from empirical studies demonstrating a negative relationship between acute stress-induced levels of CORT and measures of fitness, presumably due to a reallocation of resources from reproduction and maintenance to immediate survival (Bonier et al. 2009 a, b). This hypothesis assumes that the stress response is variable among individuals, but consistent within an individual, and predicts that individual variation in the stress response will relate to fitness. Specifically, it suggests that individuals who secrete more CORT in response to a stressor or are less able to clear CORT after a stressor dissipates will incur higher costs of elevated CORT and have reduced fitness. Therefore, it predicts lower reproductive success and/or lower survivorship among individuals with higher CORT response to an acute stressor.

A review by Breuner et al. (2008) found contradictory evidence for the CORT-fitness hypothesis among the several studies that have examined the acute stress response and fitness. They classified studies into three main categories: i) regulation of secretion, ii) performance measures, and iii) fitness effects. Studies examining the regulation of secretion, or how certain conditions alter the acute stress response and the expected fitness correlates, were for the most part in line with the CORT-fitness hypothesis. Most studies showed some sort of trade-off between reproduction and survival which is mediated by glucocorticoids. An example of this trade-off can be seen in white-crowned sparrows (*Zonotrichia leucophrys*), where individuals with poor body condition had greater glucocorticoid reactivity, an indication that when presented with a stressor they show a more pronounced shift from reproductive capacity to survival mode (Breuner & Hahn 2003). In studies examining

performance measures, or how an individual activates or suppresses certain behaviours or physiological processes in response to acute stressors, many different trends were seen. Glucocorticoids were again shown to mediate a trade-off between reproduction and survival in some species such as the white-crowned sparrow where greater glucocorticoid reactivity came at a cost to reproduction (Breuner & Hahn 2003). However, in other species such as the red-sided garter snake (*Thamnophis sirtalis parietalis*) glucocorticoid increase had no effect on reproductive behaviour (Moore et al. 2002), while in the Galapagos marine iguana (*Amblyrhynchus cristatus*; Berger et al. 2005), the musk shrew (*Suncus marinus*; Schmil & Rissman, 1999), and the cane toad (*Bufo marinus*; Orchinik et al. 1988) increased levels of glucocorticoids were found to be associated with increased reproduction at a potential cost to immunity and/or survival. Finally, the relationship between acute glucocorticoid response and direct fitness effects seems to support the CORT-fitness hypothesis in that increasing stress reactivity correlates with a shift from reproductive capacity to survival. However, in the two studies examined in this review paper that directly measured survival, the opposite pattern was seen: individuals with high reactivity had low survival. This pattern has also been shown in our population of song sparrows (*Melospiza melodia*) in the past (MacDougall-Shackleton et al. 2009). Bonier et al. (2009a) outlines clearly the theoretical tenets of the CORT-fitness hypothesis when looking at baseline levels of glucocorticoids. Theoretically, an increase in environmental challenges should lead to a corresponding increase in baseline glucocorticoids and as environmental challenges and baseline CORT increase, fitness of the individual decreases. However, in their review of the literature, they found most of the empirical evidence did not support the CORT-fitness hypothesis and its predictions in relation to baseline glucocorticoids.

Collectively, the review findings provide tentative support for some aspects of the CORT-fitness hypothesis. However, there are several occurrences where the CORT-fitness hypothesis is not supported fully in the literature. Bonier et al. (2009a) discuss reasons why empirical data may not support theory. The majority of these studies used only a single stress response per animal, essentially assuming that CORT responses are consistent (repeatable) within individuals. If CORT levels are to be used in order to predict fitness as suggested by the CORT-fitness hypothesis, then the response to acute stress should be a repeatable, consistent trait within an individual. Thus it is important to determine whether this is the case in free-living populations of animals (Cockrem & Silverin 2002, Wada et al. 2008). In birds, although maximum CORT response has been found to be repeatable in wild incubating adelic penguins (*Pygoscelis adeliae*; Cockrem et al. 2009), captive zebra finches (*Taeniopygia guttata*; Wada et al. 2008), and captive great tits (*Parus major*; Cockrem & Silverin 2002), baseline CORT is highly dependent on prior activity and may be less repeatable (Romero & Reed 2008, Cockrem et al. 2009). As well, changes in life history stage may be associated with variation in the relationship between CORT and fitness. An increase in maternal CORT altered the offspring sex ratio by increasing the number of females in white-crowned sparrows (Bonier et al. 2007). Under different environmental situations higher levels of glucocorticoids may result in different behaviours. Astheimer et al. (1992) found when white-crowned sparrows were provided with unlimited access to food, individuals with glucocorticoid implants were less active, however the reverse was seen when individuals were food restricted. Also, to date, only a few empirical studies have examined the relationship between the increase in glucocorticoids in response to acute stressors and direct measures of fitness. Marine iguanas with higher maximum CORT levels after an acute stressor were found to have lower survivorship of an El Niño event than individuals whose CORT response was lower (Romero & Wikelski

2001). Similarly, male song sparrows with higher CORT levels in response to capture stress were less likely to return to the breeding site the following year, and were thus presumed dead (MacDougall-Shackleton et al. 2009). Finally, reproductive success in breeding black-legged kittiwakes (*Rissa tridactyla*) was found to be lower in individuals with a higher stress response (Angelier et al. 2009).

Importantly, the studies reviewed above (Romero & Wikelski 2001, Angelier et al. 2009, MacDougall-Shackleton et al. 2009) examined only baseline and/or maximum CORT, not the functioning of the entire HPA axis. No published studies have yet addressed the regulation of the HPA axis and how it relates to fitness in the wild. The CORT-fitness hypothesis proposes that an adaptive stress response involves not only rapid secretion of CORT when the stressor is encountered, but also rapid clearing of CORT from the system once the stressor has been dealt with. Therefore, when relating stress response and fitness it is important to characterize the entire response. When studying wild animals, the stressor itself (usually captivity stress) cannot be removed while continuing to monitor the subject. However, exogenous hormones can allow HPA functioning to be examined. First, dexamethasone (DEX) is an analogue to CORT that binds to glucocorticoid receptors and inhibits the release of HPA axis hormones via negative feedback. Therefore, the use of DEX allows us to assess the efficiency of an individual's negative feedback mechanisms. Second, ACTH also can be administered exogenously to experimentally determine the sensitivity of the adrenal cortex and resulting CORT production. These methods have been used to characterize the HPA axis functioning in chukars (*Alectoris chukar*; Dickens et al. 2008). Thus, ACTH and DEX can be used to characterize individual variation in HPA function, including adrenal sensitivity to ACTH, as well as sensitivity of the HPA axis to negative feedback.

Song sparrows (*Melospiza melodia*) are an ideal species in which to study HPA regulation and fitness. On property owned by the Queen's University Biology

Station, we have access to a population of song sparrows that have been studied and monitored since 2002. Greenwood and Harvey (1982) examined song sparrow return rates and found song sparrows to be highly philopatric. Our study population has a 40-60% overwinter return rate in adults, and individuals typically return to breed within approximately 75 m of their previous territory (E. A. MacDougall-Shackleton, unpublished data). Because of their high degree of territoriality, males are easy to capture using song playback and mist-nets. Being able to quickly capture the individuals is imperative for studying stress responses as plasma CORT levels increase within about 3 minutes of disturbance (Wingfield et al. 1992, Rich & Romero 2005). Many different measures of fitness and reproduction can be collected from focal individuals and their offspring, and these measures can often be repeated and compared in subsequent seasons. As well, previous stress response studies have been carried out on our population, which allow for comparison between years. Similar results between years could point towards stress response and fitness relationships in our population remaining stable over seasons, whereas different results could indicate a more dynamic relationship between stress and fitness and perhaps fluctuating selection pressures acting on the population.

I tested the CORT-fitness hypothesis in males from this free-living population of song sparrows. The CORT-fitness hypothesis predicts that individual variation in the HPA response to stress is related to individual variation in fitness. Specifically, it predicts that individuals who secrete more CORT in response to a stressor, or are less able to clear CORT after a stressor dissipates, will incur higher costs of elevated CORT and have reduced fitness. Thus, I predicted lower reproductive success and/or lower apparent survivorship (estimated by overwinter return rates) among individuals with higher CORT response to an acute stressor (capture and restraint), individuals with higher CORT response to exogenous ACTH, and individuals with less CORT suppression in response to DEX.

Chapter 2: MATERIALS AND METHODS

Pilot Study

In order to determine the time course of the adrenocortical response in song sparrows, and the optimal dose and timing for DEX and ACTH challenges, I conducted pilot studies using wild-caught song sparrows. I captured adult male song sparrows (n= 12) near the University of Western Ontario (43°98'N, 81°24'W) using a mist-net and song playback in August and September 2009. Birds were handled and cared for in accordance with UWO animal care guidelines (Animal Care Protocol 2007-089). They were caged individually on natural light schedules until fall moult was complete (approximately 2 months). Birds were fed budgie seed mix ad libitum. In early November, birds were switched to a long day photoperiod (14 h L : 10 h D) to induce reproductive condition. I then conducted a series of tests to assess response to stress, ACTH and DEX after three weeks on the long day photoperiod. Birds were allowed to recover for a minimum of 7 days between subsequent tests. All tests were concluded by mid-December 2009.

Stress Response: The stress response test followed a standard restraint protocol widely used in a number of bird species (Wingfield et al. 1994). Birds were removed from their home cages and blood samples of 50 μ L were collected into heparanized microhematocrit tubes following brachial venipuncture with a 26 gauge needle within 3 minutes of the researchers entering the room (*baseline*). Following the initial blood sample, birds were then placed into individual opaque cloth bags for 10 minutes to elicit a stress response. After 10 minutes a second 50 μ L blood sample was collected (*time 10*), and the birds were returned to the bags for an additional 20 minutes. At this time a final 50 μ L blood sample was collected (*time 30*).

ACTH and Dexamethasone Dosing Studies: Baseline blood samples (25 μ L) were collected within 3 minutes of the researchers entering the room. Subjects were then given a pectoral intramuscular injection of either 25 IU/kg (n = 6) or 100 IU/kg (n = 6) of adrenocorticotrophic hormone (ACTH; Sigma Aldrich, A6303), or 0.5 mg/kg (n = 5) or 1.0 mg/kg (n = 5) of dexamethasone (DEX; Sandoz Canada Inc, 2301) dissolved in lactated Ringer's solution. They were then individually placed into opaque cloth bags as outlined above to elicit a stress response. 25 μ L blood samples were collected at 15, 30, and 60 minutes for ACTH and 30, 60, and 90 minutes for DEX to assess changes in plasma CORT concentrations.

Hormone Assay: Following blood collection, samples were kept cool, then centrifuged for 10 minutes at 13,000 g, and then the supernatant plasma was collected with a Hamilton syringe and frozen until assay. CORT was measured in unextracted plasma using an enzyme immunoassay (Assay Designs, 900-097) that has previously been validated in other sparrow species (Wada et al. 2007).

Data Analysis: Independent t-tests were run to determine differences between doses of ACTH and DEX. Pilot data were then pooled by hormone treatment and t-tests were run to determine differences in plasma CORT levels.

Field Study

Capture Protocol: Research was conducted on land owned by the Queens University Biological Station near Newboro, Ontario (44°38'N, 76°20'W). I captured 36 adult male song sparrows using mist-nets and song playback during the pre-breeding season (April 8 – April 22) 2010. This period corresponds approximately to territory establishment, pair formation, and the initiation of nest construction. All males were captured between (06:00 and 12:00).

I assessed response to restraint stress, ACTH and DEX in one session following a protocol used in starlings and chukar (Rich & Romero 2008, Dickens et al. 2009) with time course and doses established based on my pilot study (above).

As outlined above, I collected blood samples (25 μ L) into heparanized microhematocrit tubes within 3 minutes of the bird hitting the net (*baseline*; Romero & Reed 2005) (n=36). Following the initial blood sample, birds were individually placed into opaque cloth bags for 30 minutes to elicit a stress response. After these 30 minutes a second (25 μ L) blood sample was collected (*time 30*) (n=35). Subjects were then given a pectoral intramuscular injection of DEX (1.0 mg/kg) and placed into a covered cage with access to seed and water. Sixty minutes later a third blood sample (25 μ L) was collected to assess CORT suppression by negative feedback (*time 90*) (n=36). Birds were then given a pectoral intramuscular injection of ACTH (25 IU/kg) and placed back into the covered cage. After 30 minutes, a final blood sample (25 μ L) was collected to assess adrenal sensitivity (*time 120*) (n=35). Birds were given dilute apple juice in between bleeds to prevent dehydration. Birds were released at the site of capture after morphometric measurements (see below) had been collected. DEX and ACTH were kept frozen until the day of use.

Morphometric measurements: Following blood collection I measured wing length as unflattened wing chord to the nearest 0.1 mm using dial calipers. I also measured tarsus length to the nearest 0.1 mm using dial calipers. I used a 30 g spring scale to measure mass to the nearest 0.2 g.

Hormone Analysis: Blood was kept cold in the field on ice until it could be centrifuged at the end of each day. Blood was centrifuged at 12,700 g for 10 min, and then supernatant plasma was collected, frozen and brought back to the University of Western Ontario for analysis. Corticosterone was measured using a sensitive and specific radioimmunoassay (immuChem 07-120103; MP Biomedicals, Orangeburg, NY) previously validated for song sparrow plasma (Newman et al. 2008). All samples were assayed in duplicate. Sensitivities of the assays were 1.95, 2.63, and 1.8 ng/mL, calculated as 3 standard deviations from B_0 . Within-assay coefficients of

variation for low and high controls were 16% and 9% for the first assay, 8.8% and 0.2% for the second assay, and 3.5% and 2.5% for the third assay.

Fitness Measurements: I monitored breeding activity of the birds during May and June 2010 to estimate reproductive success. Specifically I examined relative lay date (date of first egg – average date of first egg for population) as earlier nests are more likely to succeed; Brown and Brown (1999) (n=23), clutch size (i.e. number of eggs laid in the nest) (n=23), and proportion of eggs hatched (run on the subset of males whose nests successfully hatched). In 21 of 23 cases, nests were discovered after egg-laying was complete, so lay date could not be observed directly. For these cases I estimated lay date by subtracting 10 days for incubation plus 1 day per egg from the observed hatch date (Arcese et al. 2002). Since territory quality directly relates to these fitness components, and males find and defend their territory, these fitness components are appropriate to relate to male stress response. Although our population of song sparrows is multibrooded, there is limited success of secondary and tertiary nests (E. A. MacDougall-Shackleton, unpublished data).

I also measured return rates of adult males to the breeding site in spring 2011 (returned birds, n=16). This population (and this species in general) is highly philopatric (40-60% overwinter return rate for adults in the study population), and individuals return to the breeding site with high fidelity in successive years to breed. Estimated territory movement between years is <75 m (E. A. MacDougall-Shackleton, unpublished data). Individuals who did not return to the breeding season in 2011 were presumed dead (Greenwood & Harvey 1982, MacDougall-Shackleton et al. 2009).

Data Analysis: Previous studies differ in assessing either absolute levels of baseline and stress-induced CORT (which bind to different receptors; Romero 2004) or changes in CORT. Assessing absolute levels of CORT provides information regarding potential effects of CORT on receptors, however assessing changes in

CORT provides information regarding potential regulation via negative feedback or adrenal sensitivity. I thus chose to compare fitness measures to both absolute CORT levels and changes in CORT. Three CORT measures were used to assess changes in CORT: stress response (time 30 – baseline), DEX response (time 90 – time 30), and ACTH response (time 120 – time 90).

In order to make sure the protocol was effective, I ran a repeated measures ANOVA to ensure plasma CORT levels changed across the four time periods. I used multiple regressions to determine whether the various CORT measurements predicted components of reproductive success: relative lay date, clutch size, or proportion of eggs hatched. Body condition ($n=33$) was included as a covariate and was calculated as mass/tarsus as this measure is predictive of overwinter survivorship in the study population (Y. Sarquis Adamson, unpublished data). An arcsine transformation was performed on proportion of eggs hatched to normalize the data, and CORT measures were log transformed to reduce positive skew. I used binary logistic regression to determine whether my measures of stress predicted overwinter local survivorship as well as to identify any possible relationship between lay day and hatch success. SPSS 18.0 was used for all statistical analyses. All statistical tests were two-tailed.

Chapter 3:

RESULTS

Pilot Study

There were no significant differences in CORT levels between low versus high doses of either DEX or ACTH (Table 3.1). Therefore, I pooled low and high dose groups to compare CORT levels across drug treatments. Compared to 30 minutes post-restraint, CORT levels were significantly reduced 30 minutes post-injection of DEX ($t_9 = 2.549$, $p = 0.031$) and were significantly increased 30 minutes post-injection of ACTH ($t_{10} = -2.248$, $p = 0.048$) (Figure 3.1). During the stress response trial, CORT was higher after 30 minutes restraint than after 10 minutes restraint, thus 30 minute restraint was used for the field study. CORT was most reduced 90 minutes post-injection of the high dose of DEX, however I selected a collection time of 60 minute post-injection of the high dose of DEX for the field study to minimize total handling time of the bird. CORT was most elevated 30 minutes post-injection of the low dose of ACTH and this was used for the field study.

Field Study

There was a significant difference in CORT over the 4 time periods, ($F_{3,34} = 27.9$, $p < 0.0001$) indicating that CORT values changed over time (Figure 3.2). Post-hoc tests (LSD) indicated that baseline CORT was significantly lower than each of the 30 ($p < 0.0001$), 90 ($p < 0.0001$), and 120 ($p < 0.0001$) minute samples. The 30 minute CORT levels were significantly higher than the baseline and 90 minute samples ($p = 0.007$), but not significantly different than the 120minute sample ($p = 0.448$). CORT levels were lower for the 90 minute sample than the 120 minute sample, although not significantly so ($p = 0.066$).

Stress Response

Baseline CORT levels were not significantly correlated with any other stress measures. Because of high correlations between CORT levels at times 30, 90, and

Table 3.1. No significant differences in plasma corticosterone levels were detected in song sparrows injected with 25 IU/kg or 100 IU/kg of adrenocorticotrophic hormone after 15, 30, or 60 minutes of being held in opaque cloth bags. Similarly, no plasma corticosterone differences were seen in song sparrows injected with either 0.5 mg/kg or 1.0 mg/kg of dexamethasone after 30, 60, or 90 minutes of being held in opaque cloth bags.

Treatment	Time	Dose	n	Mean CORT (ng/ml)	t-stat	Significance (p)
ACTH	15	25 IU/kg	6	42.67	$t_{10} = 1.324$	0.215
		100 IU/kg	6	34.14		
	30	25 IU/kg	6	62.26	$t_{10} = 0.61$	0.555
		100 IU/kg	6	53.19		
	60	25 IU/kg	6	47.34	$t_{10} = -0.468$	0.65
		100 IU/kg	6	53.3		
DEX	30	0.5 mg/kg	5	16.32	$t_8 = -0.442$	0.67
		1.0 mg/kg	5	19.83		
	60	0.5 mg/kg	5	26.31	$t_8 = 1.459$	0.183
		1.0 mg/kg	5	12.72		
	90	0.5 mg/kg	5	12.43	$t_8 = 0.433$	0.676
		1.0 mg/kg	5	10.06		

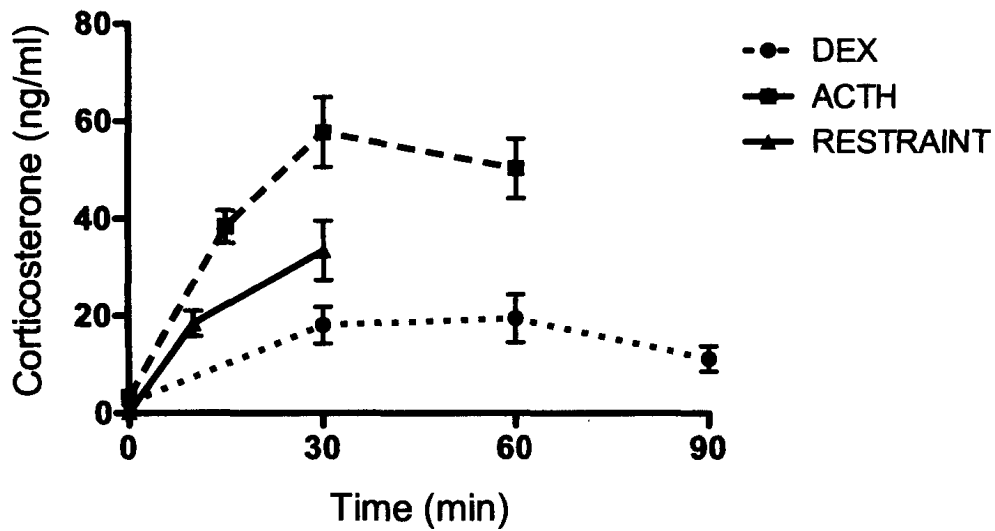


Figure 3.1 Pilot study data conducted on captive song sparrows showed significantly increased levels of plasma CORT in response to ACTH and significantly decreased levels of plasma CORT in response to DEX at 30 minutes when compared to restraint (± 1 SE) All individuals had an increase in plasma CORT from baseline as a result of capture and restraint handling.

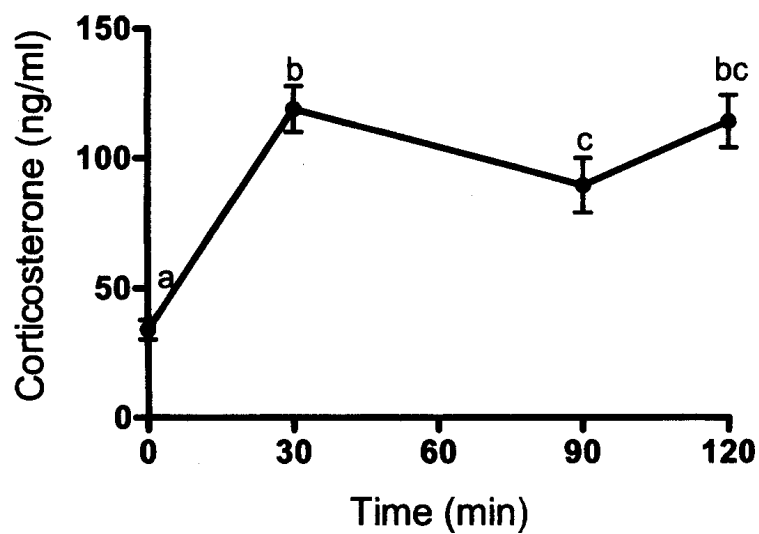


Figure 3.2 Mean plasma CORT levels (± 1 SE) across four bleed times during a field study on free-living song sparrows. Time 0 differed significantly from all other times, and time 30 differed from time 90. Although time 90 did not differ significantly from time 120 there was a strong trend towards lower plasma CORT at time 90 than at time 120.

120 (data not shown), I analyzed response (i.e. stress response, DEX response, ACTH response) in addition to these raw data. Stress-induced CORT response (time 30 - baseline) was significantly and positively correlated with CORT levels at time 30 ($r = 0.892$, $p < 0.001$), time 90 ($r = 0.503$, $p = 0.001$), and time 120 ($r = 0.392$, $p = 0.015$). ACTH-induced CORT response (time 120 - time 90) was significantly and positively correlated with DEX-induced CORT response (time 90 - time 30) ($r = 0.435$, $p = 0.006$) indicating that individuals more sensitive to negative feedback were also more sensitive to adrenal stimulation.

HPA Function and Reproduction

Lay date was negatively and significantly related to CORT level at time 30 ($\beta = 0.713$, $p = 0.005$) although not by CORT level at baseline, ($\beta = -0.209$, $p = 0.420$), time 90 ($\beta = -0.221$, $p = 0.412$), or time 120 ($\beta = -0.058$, $p = 0.167$; overall model $F_{5,20} = 3.284$, $r^2 = 0.523$, $p = 0.034$). Males with higher CORT following restraint fathered nests that had later lay dates (Figure 3.3). Absolute CORT values were not significantly related to clutch size at baseline ($\beta = -0.101$, $p = 0.724$), time 30 ($\beta = 0.026$, $p = 0.919$), time 90 ($\beta = 0.060$, $p = 0.843$), or time 120 ($\beta = -0.402$, $p = 0.214$; overall model $F_{5,21} = 0.727$, $r^2 = 0.185$, $p = 0.613$) or proportion of eggs hatched at baseline ($\beta = 0.715$, $p = 0.111$), time 30 ($\beta = -0.081$, $p = 0.831$), time 90 ($\beta = -0.344$, $p = 0.482$), or time 120 ($\beta = -0.344$, $p = 0.499$; overall model $F_{5,15} = 1.669$, $r^2 = 0.455$, $p = 0.229$).

The response to DEX significantly related to lay date ($\beta = -0.654$, $p = 0.011$). However changes in CORT were not related to lay date due to restraint only ($\beta = 0.094$, $p = 0.661$) or ACTH ($\beta = -0.423$, $p = 0.061$; overall model $F_{4,20} = 4.102$, $r^2 = 0.506$, $p = 0.018$). Individuals who exhibited a larger decrease in CORT following DEX injection (a larger negative value) fathered nests with a later lay date (Figure 3.4).

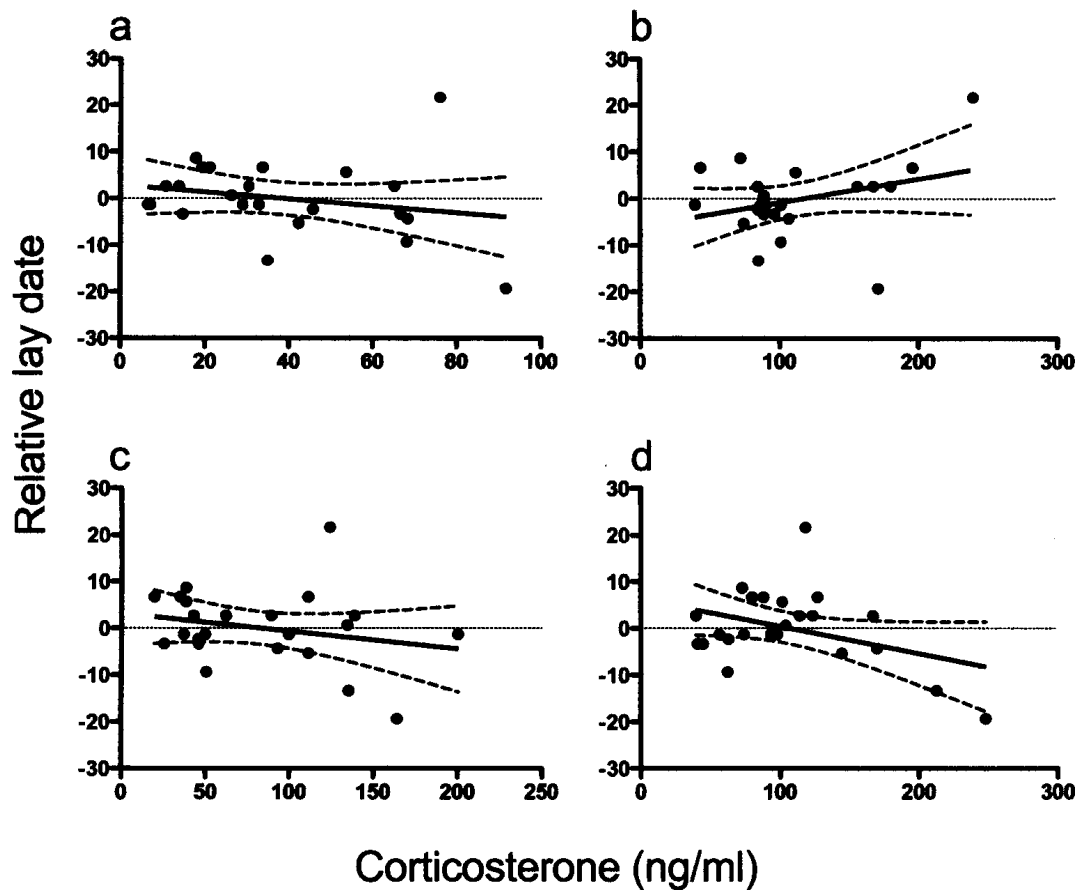


Figure 3.3 Predictive relationship of absolute plasma CORT levels and relative lay date in free-living song sparrows. a) Baseline CORT levels were not significantly predictive of relative lay date. b) CORT levels after 30 minutes of restraint only were significantly predictive of relative lay date. Individuals who had higher CORT levels after 30 minutes were more likely to have later nests. c) Absolute CORT levels taken 60 minutes after injection with DEX (time 90) were not predictive of relative lay date. d) Absolute CORT levels taken 30 minutes after injection with ACTH (time 120) were not predictive of relative lay date. Simple linear relationships are depicted; however statistical analysis included body condition as a covariate. Dashed lines indicate 95% confidence intervals of the regression line.

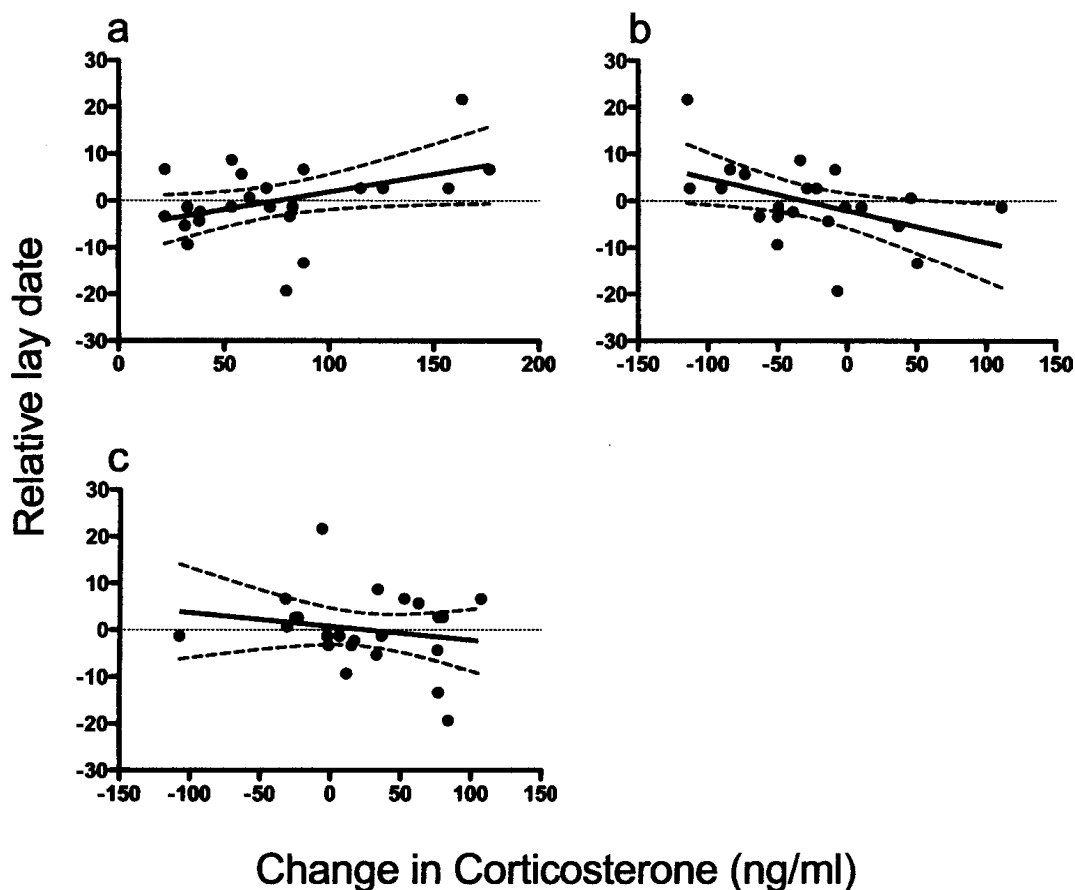


Figure 3.4 Predictive relationship between changes in plasma CORT levels and relative lay date in free-living song sparrows. a) The change in plasma CORT levels as a result of restraint (time 30 – time 0) was not significantly predictive of relative lay date. b) The change in CORT as a result of injection with DEX (time 90 – time 30) was significantly predictive of relative lay date. Individuals who had a larger decrease in plasma CORT in response to DEX injection had later nests. c) The change in CORT as a result of injection with ACTH (time 120 – time 90) was not predictive of relative lay date. Simple linear relationships are depicted; however statistical analysis included body condition as a covariate. Dashed lines indicate 95% confidence intervals of the regression line.

Changes in CORT did not significantly relate to clutch size due to restraint ($\beta = -0.115$, $p = 0.680$), DEX ($\beta = -0.238$, $p = 0.455$), or ACTH response ($\beta = -0.383$, $p = 0.181$; overall model $F_{4,21} = 0.594$, $r^2 = 0.123$, $p = 0.672$) or proportion of eggs hatched due to restraint ($\beta = -0.725$, $p = 0.042$), DEX ($\beta = -0.651$, $p = 0.048$), or ACTH response ($\beta = -0.061$, $p = 0.832$; overall model $F_{4,15} = 1.935$, $r^2 = 0.413$, $p = 0.175$). Combined, these results indicate that birds who had the highest levels of CORT following restraint, and/or decreased CORT the most following DEX injection, had later lay dates.

Stress and Survival

Logistic regressions indicated no significant predictive value for absolute CORT values (Wald = 9.115, $n = 32$, $p = 0.105$), or stress response measures (Wald = 6.792, $n = 32$, $p = 0.147$) on the likelihood of overwinter survival (Figure 3.5). Thus, in contrast to prior research, none of my measures of HPA regulation predicted overwinter survival.

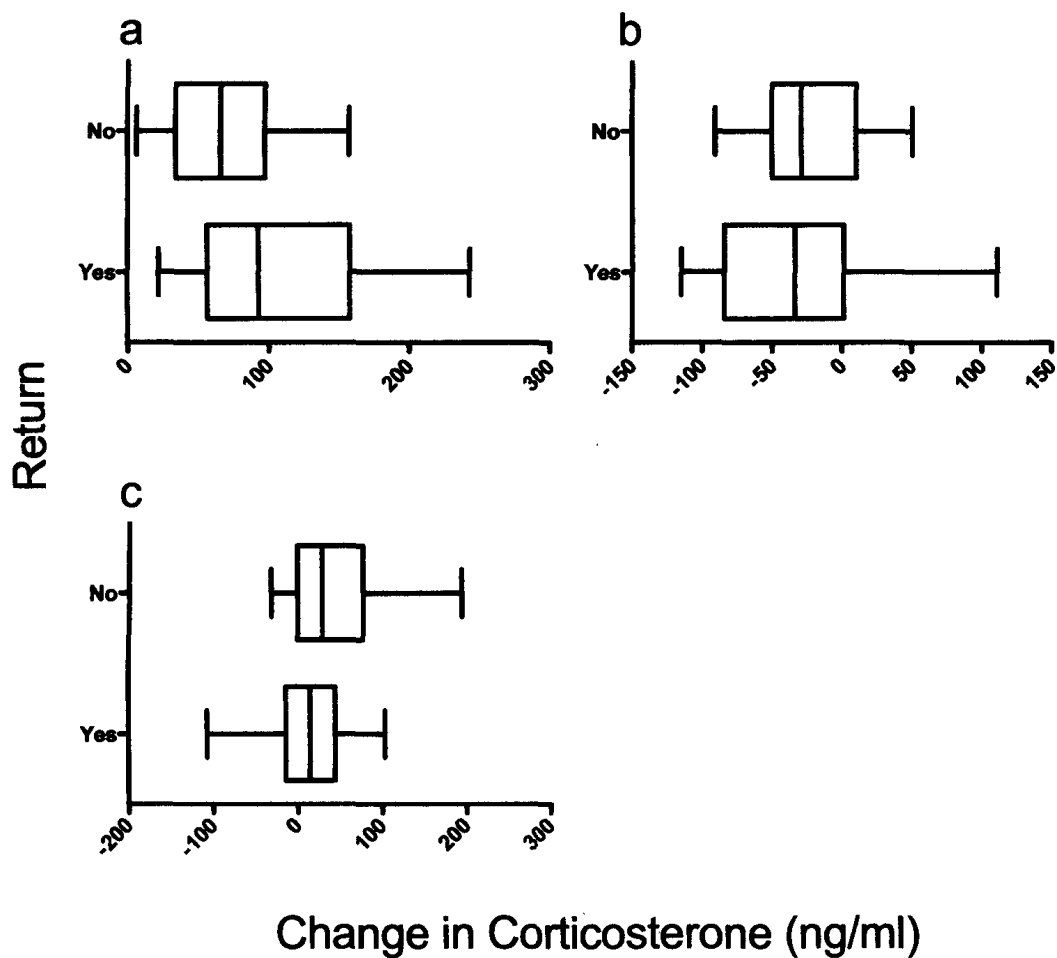


Figure 3.5 Changes in plasma CORT levels due to stress response measures were not significantly predictive of return rate in our population of free-living song sparrows for a) natural change in CORT as a result of restraint only (time 30 – time 0), b) change in CORT as a result of injection with DEX (time 90 – time 30), or c) change in CORT as a result of injection with ACTH (time 120 – time 90).

Chapter 4:

DISCUSSION

The results of this study provide mixed support for the CORT-fitness hypothesis in song sparrows. Interestingly, individuals who were more sensitive to DEX injection also tended to be more sensitive to ACTH injection, as subjects who decreased plasma CORT more in response to DEX also increased CORT more in response to ACTH. Stress measures were not significantly predictive of clutch size, proportion of eggs hatched, or overwinter return rate. However, plasma CORT levels 30 minutes after restraint and change in plasma CORT levels as a result of exogenous DEX injection were significantly predictive of lay date. Individuals with higher level of plasma CORT 30 minutes after restraint, and individuals with larger decreases in CORT in response to DEX, were more likely to have later nest dates.

As described earlier, the receptors targeted by ACTH and DEX are located in different regions of the body. DEX, as a CORT agonist, targets mineralcorticoid and glucocorticoid receptors throughout the body, while ACTH targets receptors in the adrenal glands. The positive relationship between the sensitivity of these receptors supports the predictions of the CORT-fitness hypothesis, that individuals should have consistent variation in their responsiveness to stressors. This could indicate that individuals are consistent in their sensitivity to various hormones in the HPA axis. This relationship may exist only in the HPA axis, or it could extend to other hormone interactions as well.

Plasma CORT levels at 30 minutes post restraint and change in plasma CORT levels as a result of exogenous DEX injection were significantly and negatively related to lay date (Figure 3.3, 3.4). The relationship between stress-induced CORT and lay date is consistent with the CORT-fitness hypothesis. Birds who reacted most to the stressor had later nests, which is correlated with lower fitness (Hochachka & Smith 1991). However, birds that suppressed CORT the most following DEX

injection had later lay dates. This suggests that higher sensitivity to negative feedback is correlated to lower reproductive success. Further tests of negative feedback of unstressed birds would be required to verify this.

In free-living bird species, the timing of nesting is a particularly important aspect of breeding ecology and has been linked to plasma CORT levels in the past (Shini et al. 2009, Ouyang et al. 2011). Birds must time breeding to correspond with weather patterns, food availability, and/or migration. Most seasonally breeding birds in the temperate zone, including song sparrows, breed between mid-May and mid-June. Studies have shown that individuals who have an earlier lay date are more reproductively fit and have higher fledging success and survival of their offspring (Hochachka & Smith 1991, Brown & Brown 1999, Ouyang et al. 2011). High stress-induced plasma CORT levels, and small decrease in CORT due to DEX, corresponded with a delay in lay date when compared to average lay dates in the population. In song sparrows, a migratory species, birds must return to the breeding ground in the early spring, find and defend an adequate territory, and find a mate before beginning to breed. Low quality males have been shown to be unable to defend high quality territories (Brockleman 1975) and, many studies on song sparrows have focused on song repertoire size and fitness, indicating that individuals with smaller song repertoires are less preferred by females compared to males with large, complex song (Catchpole 1987, Hill et al. 2011). Previous research on this population of song sparrows has shown relationships between HPA functioning and song, birds with greater stress responses to capture-restraint protocol have been shown to have less complex song (MacDougall-Shackleton et al. 2009, Schmidt et al. submitted). It may take longer for individuals who are located on poorer territories and have small repertoire sizes to find a mate who is willing to "settle" for a lower quality male, thus delaying the onset of pair formation and breeding. Also, since eggs and unfledged nestlings are subject to high predation rates (approximately 50% in some years),

having an earlier lay date may allow additional time to make a second, or even third, attempt at nesting (Arcese et al. 2002). Further work is required to determine the relationships among male quality, song, HPA function, and lay date in this species.

That the stress response was only predictive of lay date in this study provides insight into the importance of pair formation and breeding timing. Since delayed breeding can have such a large impact on the success of a nest, strong selection pressures must be working to maintain appropriate timing. Individuals who fall behind in breeding are less reproductively successful, which could be related to a variety of disadvantages to late breeding. Food availability may be decreased later in the season as many individuals are feeding themselves and their offspring, less food or lower quality food may result in lower survival of nestlings. Several studies have examined nesting food deprivation and found that individuals receiving less food, or lower quality food, are less fit as adults and more prone to parasitism and illness (Bize et al. 2010). This also seems to correspond with song development; individuals who were subjected to food restriction early in development tend to have smaller song repertoires as adults (Spencer et al. 2004). Thus, there are many potential advantages to breeding early, and individuals who bred early would be expected to have a lower stress response to capture-restraint protocol.

Clutch size is frequently used as a measure of reproductive success, individuals who are able to have and care for larger clutches, tend to have higher reproductive success. No predictive relationship was seen between stress response measures and clutch size in our population. This is in contrast with the CORT-fitness hypothesis that predicts individuals with higher levels of baseline glucocorticoids should have smaller clutch sizes. Song sparrows typically lay 1 egg per day for 4-5 days, resulting in a clutch size of 4-5. In our population, there is very little variation in the number of eggs produced by each female (range 3 to 5 eggs). Due to such low variation, it would be difficult to detect a relationship between clutch size and any other

variable. Additionally, I examined the relationship between stress and the proportion of eggs hatched. As mentioned earlier, predation rates are high in our population, so in order to control for this, I analyzed the proportion of nestlings hatched only in nests that were not subject to predation. Stress was not predictive of the proportion of nestlings hatched in our population. This emphasizes the fact that predation is the driving factor in nest success. Other measures of reproductive success may thus be more appropriate when examining the CORT-fitness hypothesis.

Song sparrows are highly philopatric and return to breed at approximately the same location each season, individuals who do not return are presumed dead. In previous seasons, stress response has been predictive of return rates (MacDougall-Shackleton et al. 2009). Individuals who had greater increases in plasma CORT after 30 minutes of restraint were less likely to return the following season. In contrast to this prior study, stress response measures had no predictive relationship with return rate after the winter of 2010-2011. This finding could indicate a shift in selection pressures, wherein variation of stress response was not related to survival. In previous seasons, the optimal stress response may have been different, where a less reactive stress response was favored (MacDougall-Shackleton et al. 2009). A dynamic selection pressure, varying from year to year, could be one mechanism for maintaining variation in the stress responses of populations. Our population of song sparrows are believed to overwinter in the southeastern United States. Average weather data compiled by the NOAA National Climate Data Center indicates reduced average temperature and precipitation in the southeastern US in 2010-2011 compared to 2007-2008. From December to March 2007-2008 average temperature was 50.7°F and average precipitation 15.46". For the same months in the 2010-2011 season, average temperature fell to 47°F and average precipitation dropped to 13.19" (Enloe 2011). Annual variation in weather conditions could affect

populations directly and indirectly. Direct effects of weather could include higher amounts of rain, thereby reducing the amount of time individuals are able to spend foraging and reductions in temperature could result in more energy being allocated to maintaining a core body temperature. Indirect effects of weather conditions include changes in food availability and changes in predator/prey populations. The direct and indirect influences of weather conditions provide enough variation in selection pressures to change optimal stress responses for these two seasons.

Further research needs to examine the relationship between HPA regulation and fitness in females, as this study only looked at male fitness. Female CORT levels have been shown to have profound effects on egg and offspring development (Hayward & Wingfield 2004, Meylan & Clobert 2005, Eriksen et al. 2006). Additionally, this study was performed over a single year, in order to fully understand relationships between stress and fitness, long term studies need to be conducted. This will allow researchers to see potential variation in the relationship between stress and fitness; in philopatric species such as the song sparrow, this variation in success may even be measured in the same individual. A long-term study will also provide researchers with a means of measuring annual repeatability, which could indicate the response to stress is a consistent trait within an individual. Finally, due to separate research performed on nestlings, I was not able to assess all nestling fitness measurements in this study (i.e., fledging success, nestling growth rate, nestling CORT profiles). Further research on nestling growth and other performance measures would provide us with further evidence of the direct fitness consequences of varying stress responses.

This study examined the relationship between HPA regulation and fitness in an attempt to test the CORT-fitness hypothesis. The results provided mixed results for the applicability of the CORT-fitness hypothesis. Although lay date was significantly predicted by plasma CORT levels at time 30 and in response to DEX, other fitness

measures were not predicted by stress measures. Because greater reactivity has been shown to vary in its relationship with fitness, perhaps variation of this trait is maintained through shifting selection pressures each season. Thus, although one year it may be beneficial to have a highly reactive stress response, in other years it may be the opposite, or even make no difference at all. Research into hormone regulation should look further into sensitivity correlations among various hormone mechanisms to see if this is a consistent trait within individuals.

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APPENDIX 1



Date: 09/29/2008

Dear Dr. MacDougall-Shackleton:

An ANIMAL NUMBER ADJUSTMENT to your "Animal Use Protocol" #2007-089-09 entitled **Stress, development and the avian brain** has been approved.

These strains and animal numbers are approved from 06/18/2008 until the protocol full expiry date 08/31/2011.

SPECIES	STRAIN &/or OTHER SPECIES DETAIL	AGE or WEIGHT	SEX	ANIMAL NUMBER
Songbird	song sparrows	adult	M:F	144
Songbird	starling	adult	M:F	144
Songbird	song sparrow	juvenile	M:F	64
Songbird	chickadee	juvenile	M:F	64
Songbird	starling	juvenile	M:F	64
Songbird	song sparrow	adult	M:F	360
Songbird	chickadee	adult	M:F	240
Songbird	starling	adult	M:F	240
Songbird	goldfinch	adult	M:F	180

c.c. Approval Letter - S. MacDougall-Shackleton, J. Majewski

The University of Western Ontario
 Animal Use Subcommittee / University Council
 Health Sciences Centre, • London, Ontario • Canada
 PH: 519-661-2111 ext. 86770 • FL 519-661-2028 • www.uwo.ca/animal