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Variation in Parental Effort, Sexual Signaling, and the Adrenocortical Stress Response

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Variation in Parental Effort, Sexual Signaling, and the Adrenocortical Stress Response in the Song Sparrow (*Melospiza melodia*)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

Evolution, Ecology and Organismal Biology

by

Melissa Lin Grunst

December 2013

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--*Was wir alleine nicht schaffen, das schaffen wir dann zusammen*, Xaiver Naidoo

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I dedicate this dissertation to my family and to the sparrows.
Wunderbare Tiere, nicht wahr?
Franz Kafka

ABSTRACT OF THE DISSERTATION

Variation in Parental Effort, Sexual Signaling, and the Adrenocortical Stress Response in the Song Sparrow (*Melospiza melodia*)

by

Melissa Lin Grunst

Doctor of Philosophy
Graduate Program in Evolution, Ecology and Organismal Biology
University of California, Riverside, December 2013
Dr. John T. Rotenberry, Chairperson

Individuals may adjust parental effort with respect to the value of the current brood versus future reproductive potential, and the adrenocortical stress response may mediate parental allocation decisions by diverting energy investment towards self-maintenance. In this dissertation, I test the hypotheses that the stress response negatively correlates with elaboration of the sexually selected trait of song complexity and with parental effort, that paternal and maternal effort correlate with song complexity due to the signaling function of song, and that predation risk may induce relationships between song complexity, the stress response, and parental effort by elevating costs of parental care. I used song sparrows (*Melospiza melodia*) as a model species. Song sparrows are monomorphic, but males alone sing a complex, sexually selected song. I recorded nesting and singing behavior, assessed parental responses to experimentally elevated perceived predation risk, and measured the stress response following capture-induced stress. Additionally, to assess whether modulation of the stress response may help organisms adapt to anthropogenic disturbance, I investigated differences in stress physiology and condition within an urban song sparrow population. I found that two components of song complexity correlated distinctly with the stress response and maternal and paternal effort, suggesting that song may fulfill a multiple messaging function, and challenging the idea that negative correlations between the stress response and elaboration of sexual ornaments are established during development. Further, predation risk induced relationships between both song traits and the stress response and some metrics of maternal and paternal effort, suggesting that predation risk may modify the extent to which song signals paternal benefits, maternal allocation on the basis of song traits, and fitness ramifications of differences in stress physiology. On the inter-individual level, I found little evidence that smaller stress responses translate into higher parental effort. Indeed, paternal effort positively correlated with the stress response, perhaps because males with larger stress responses are well prepared to evade predation risk. Finally, song sparrows breeding in areas with high anthropogenic disturbance levels did not suffer declines in condition and tended to have smaller stress responses, suggesting that sparrows successfully cope with selective pressures unique to urban areas.

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Variation in Parental Effort, Sexual Signaling, and the Adrenocortical Stress Response in the Song Sparrow (*Melospiza melodia*)

GENERAL INTRODUCTION

Given finite resources, organisms face a tradeoff between investing in reproduction and parental care versus survival and somatic maintenance (Williams 1966; Roff 1992). Since selection acts to optimize life-time reproductive success, life-history theory predicts that species with low survival probability and low future reproductive potential display high reproductive effort, whereas species with high survival probability and future reproductive potential display low reproductive effort (Williams 1966; Gadgil and Bossert 1970; Stearns 1976; Roff 1992). In addition, in the process of caring for offspring, species with different life-history strategies should display patterns of behavioral plasticity that reflect differential balance of the survival-reproduction tradeoff. Specifically, when confronted with offspring- and adult-directed predation risk, species with face-paced life history strategies and high brood value should respond in fashions that prioritize offspring fitness, whereas species with slow-paced life history strategies should respond in fashions that prioritize self-maintenance (Magnhagen 1990; Ghalambor and Martin 2000, 2001, 2002; Ackerman et al. 2006).

Intraspecific variation in parental effort and patterns of parental risk-taking are often discounted as noise (Sih et al. 2004; Korte et al. 2005). However, even within a species individuals may display differences in parental effort and risk-taking that have an adaptive basis. Indeed, theory predicts that individuals should adjust reproductive effort to reflect variation in the value of the current reproductive attempt, or brood, as modified by factors including individual condition or mate quality (Houston and McNamara 1992; McNamara and Houston 1992; Kawecki and Stearns 1993; Ghalambor and Martin 2000, 2002; Lima 2009). Moreover, predation risk, and other factors that elevate the costs of parental care, may accentuate differences in the parental strategies between individuals within populations (Candolin 1998; Matessi et al. 2009). Indeed, male rock sparrows (*Petronia petronia*) display higher levels of costly nest defense behaviors when mated to females with colorful sexually selected plumage traits, but do not display higher levels of less-risky, nestling provisioning behavior (Matessi et al. 2009).

In this dissertation I assess the degree to which intraspecific variation in parental effort and parental risk-taking exists in the song sparrow (*Melospiza melodia*). Specifically, I investigated whether individual differences in parental effort and risk-taking behavior are associated with differences in the expression of the sexually selected trait of song complexity and the adrenocortical stress response. The adrenocortical stress response is a physiological control mechanism that may mediate balance of the survival-reproduction tradeoff, the expression of sexually selected traits, and behavioral responses to both predation risk and anthropogenic disturbance (Wingfield and Sapolsky 2003; Husak and Moore 2008; Bonier 2012).

Sexually selected traits and parental effort and risk-taking

Diverse hypotheses have been proposed to explain how expression of sexual ornaments, including song complexity in birds (Box 1) may translate into differences in parental effort and risk-taking in both males and their female partners. In males, the good parent hypothesis (Hoelzer 1989) suggests that sexual displays serve, at least in part, as indicators of paternal ability, such that females gain paternal benefits by mating with males with elaborate sexual displays. In this case paternal effort is expected to positively correlate with the expression of sexually selected traits. Further, given a good parent process of sexual selection, the expression of sexual ornaments may also reflect a willingness to take risks for offspring (Candolin 1998; Quesada and Senar 2007). For instance, in the three-spined stickleback (*Gasterosteus aculeatus*) males with more colorful carotenoid pigmentation maintain paternal effort under predation risk, whereas less colorful males do not (Candolin 1998). Conversely, the tradeoff (Magrath and Komdeur 2003), and differential allocation hypotheses (Burley 1986, 1988) suggest that expression of sexual ornaments negatively correlate with paternal services and risk-taking. In the first case, this negative correlation is expected because high quality males with elaborate ornaments tradeoff paternal effort against mating effort, and in the second case, because females mated to high quality males increase parental effort, thus allowing the male to reduce paternal effort. For example, in the common yellowthroat (*Geothlypis trichas*) sexual plumage pigmentation of males negatively correlates with paternal services (Mitchell et al. 2007).

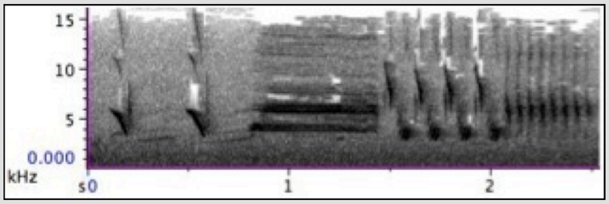
Further, in females, the differential allocation (Burley 1986, 1988) and reproductive compensation (Bluhm and Gowaty 2004; Harris and Uller 2009) hypotheses predict opposite patterns of covariation between the sexual ornamentation of males and parental effort in females. Specifically, the differential allocation hypothesis suggests that females gain fitness returns by up-regulating parental effort when mated to males with elaborate sexual displays that reflect the indirect benefits of high genetic quality. Thus, the differential allocation hypothesis predicts a positive relationship between maternal effort and risk-taking in females and male sexual ornamentation. Conversely, the reproductive compensation hypothesis (Bluhm and Gowaty 2004; Harris and Uller 2009) predicts that females mated to low quality males may increase maternal effort and risk-taking to help ameliorate fitness declines which could result as the consequence of low male genetic quality or paternal services. Thus, the reproductive compensation hypothesis predicts a negative relationship between maternal effort and risk-taking and male ornamentation. Some empirical support has been found for each of these hypotheses (Harris and Uller 2009; Ratikainen and Kokko 2010; Horvathova et al. 2012). For instance, in blue tits (*Cyanistes caeruleus*) females mated to males with bright UV coloration provision nestlings at higher rates and defend nests more vigorously than females mated to dull males

(Limbourg et al. 2004; Johnsen et al. 2005), supporting the differential allocation hypothesis. In contrast, female barn swallows (*Hirundo rustica*) increase investment of carotenoids into eggs when paired to less ornamented males, a pattern consistent with the reproductive compensation hypothesis (Saino et al. 2002).

In addition, different sexually selected traits may communicate distinct information about male genetic or phenotypic quality, as proposed by the multiple messages hypothesis (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003).

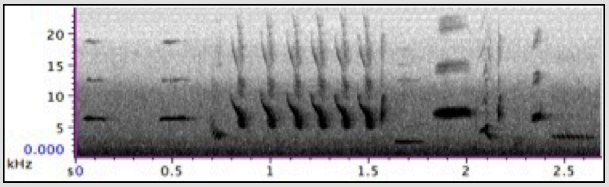
BOX 1. SONG COMPLEXITY IN *M. MELODIA*

Song type 1



Syllables 1 2 3,4 5

Song type 2



Syllables 1 2 3 4,5 6 7,8,9,10

M. melodia is monomorphic in appearance, but males alone sing. Song complexity can be measured at two levels, as depicted to the left. Firstly, males have multiple song types in their repertoire (5-12 song types in my study population). Secondly, multiple syllables exist within each song type, such that a total syllable repertoire size can also be calculated (25-43 syllable types in my population). Song complexity in *M. melodia* has been linked to female choice and numerous other fitness metrics (Searcy and Anderson 1986; Hiebert et al. 1989).

Indeed, a number of past studies have found that different components of multifaceted sexual displays communicate different information, which may help explain the elaboration of sexually selected traits (Senar et al. 2003; Quesada and Senar 2007; Taff et al. 2012). For example, in great tits (*Parus major*), the size of the black melanin-based breast stripe positively correlates with nest defense, whereas carotenoid-based plumage coloration fails to predict nest defense, but does positively predict body condition and nestling provisioning rates (Senar et al. 2003; Quesada and Senar 2007). Clearly, in the case that different sexually selected traits communicate distinct information about male paternal services or genetic quality, different dimensions of sexual displays might correlate with parental behaviors in both sexes in non-equivalent fashions.

In this dissertation I simultaneously explored the alternative hypotheses discussed above for both males and females. To this end, I measured male song complexity (a multidimensional sexually selected trait consisting of both song repertoire size and song syllable diversity, Box 1), and employed predator presentation experiments, which are discussed more thoroughly below.

The adrenocortical stress response and parental effort and risk-taking

The adrenocortical stress response helps regulate balance of the survival-reproduction tradeoff in vertebrates, and is thus expected to covary with levels of parental effort and risk-taking, as well as other variables that affect brood value. During a stress response the hypothalamus-pituitary-adrenal (HPA) axis functions to control release of glucocorticoids (corticosterone (CORT) in birds) from the adrenal cortex (Box 2, Wingfield 1994; Sapolsky et al. 2000; Wingfield and Sapolsky 2003). These steroid hormones act as transcription factors, and have wide-ranging effects including regulation of glucose metabolism, modulation of immune function, and at least after exposure to a stressor, forestalling investment into reproduction in favor of survival (Wingfield et al. 1992; Sapolsky et al. 2000; Romero 2002; Wingfield and Sapolsky 2003). Thus, down-regulation of the stress response during the breeding season may be crucial to reproductive success. However, individuals with less invested in the current brood may maintain larger stress responses as a means of facilitating self-maintenance activities in the face of environmental challenges (Wingfield and Sapolsky 2003).

Down-regulation of the stress response may be especially influential when considering maintenance of parental behaviors in the face of perturbations, which might otherwise terminate reproductive activity and induce survival behavior. Indeed, empirical evidence suggests that down-regulation of the stress response during the breeding season is more pronounced in stochastic arctic or alpine environments relative to in lower latitude or lower elevation populations (Wingfield et al. 1992; Wingfield et al. 1994a, b; Wingfield et al. 1995; Silverin et al. 1997; Silverin and Wingfield 1998; Meddle et al. 2003; Holberton and Wingfield 2003; Lynn et al. 2003; Pereyra and Wingfield 2003). Moreover, recent work in urban environments suggests a parallel pattern in which down-regulation of the stress response may help sustain breeding activity in the face of frequent anthropogenic disturbances (Partecke et al. 2006; Bonier et al. 2007; French et al. 2008; Fokidis et al. 2009; Atwell et al. 2012). Additionally, correlations between the stress response and behaviors may only arise in the context of predation risk, or other threats to survival. For instance, Lendvai and Chastel (2010) found that house sparrows (*Passer domesticus*) with higher stress-induced CORT concentrations fed offspring at lower rates after exposure to a stressor than sparrows with lower CORT levels, but that differences in adrenocortical stress physiology did not relate to baseline feeding rates.

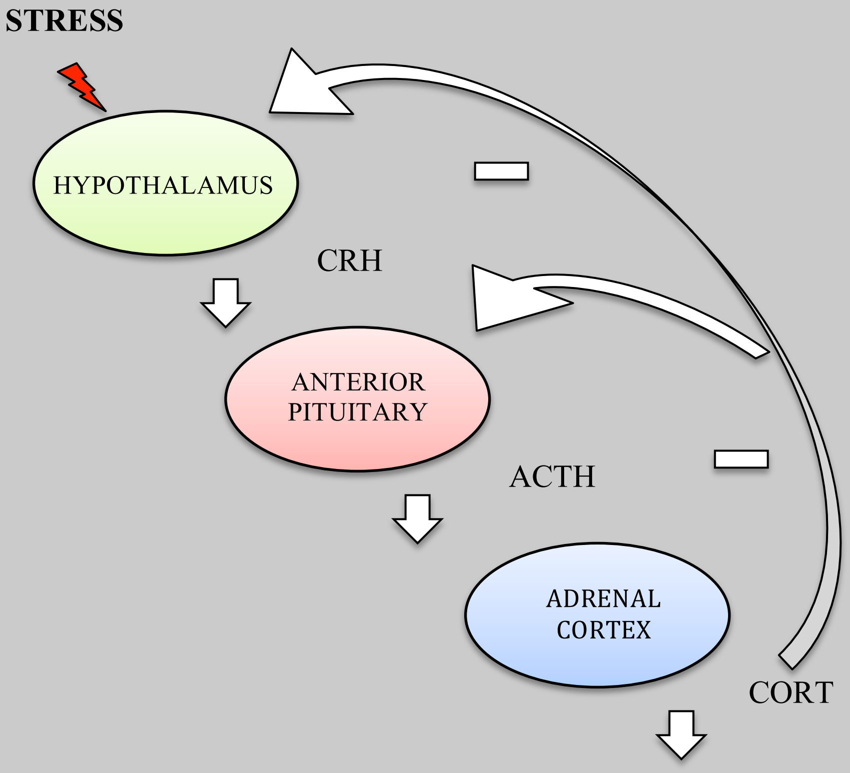
Thus, in this dissertation I test the hypothesis that individuals investing highly in reproductive activities down-regulate the adrenocortical stress response relative to individuals prioritizing survival (Romero 2002; Wingfield and Sapolsky 2003). In addition, I explore the contingency that individuals with suppressed stress responses display patterns of parental risk-taking behavior that prioritize offspring wellbeing, whereas individuals with higher stress responses prioritize survival. To this end, I measured the stress response by capturing birds in mist nests and employing standard capture protocol (Wingfield et al. 1992), and correlated variation in CORT levels to variation in parental effort and risk-taking behaviors.

In the final section of this dissertation, I examine associations between levels of anthropogenic disturbance and the stress response during the breeding season within an urban environment. Although patterns of parental effort and risk-taking were not directly assessed within the urban setting, maintaining small stress responses may be especially important to sustained reproductive activity in the face of anthropogenic disturbance, as previously suggested for species including European blackbirds (*Turdus merula*) and dark-eyed juncos (*Junco hyemalis*). Thus, documenting variation in the stress response based on differences in disturbance levels may elucidate whether changes in the HPA axis aid in adaptation to unique urban selective pressures (Partecke et al. 2006; Atwell et al. 2012; Bonier 2012).

Predator presentation experiments

To assess parental effort and risk-taking in relation to song complexity and variation in the stress response, I performed two predator presentation experiments. These experiments aimed to simultaneously assess baseline reproductive effort and responses to predation risk. Further, I was able to assess whether relationships between song complexity, the stress response and levels of parental effort might arise only in the context of predation risk, when costs of parental care are elevated.

BOX 2. THE ADRENOCORTICAL STRESS RESPONSE



The adrenocortical stress response, controlled by the hypothalamus-pituitary-adrenal (HPA) axis, is one major component of the vertebrate response to stress. During a stress response an external stressor is perceived by the hypothalamus, resulting in release of corticotropin releasing hormone (CRH). CRH then stimulates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn triggers release of steroid glucocorticoids from the adrenal cortex. Corticosterone (CORT) is the major glucocorticoid in birds, reptiles, and many mammals. Glucocorticoids enter circulation and have wide-ranging effects, including suppression of reproductive functions. CORT also acts to suppress release of CRH and ACTH at the levels of the pituitary and brain, creating a negative feed back loop, which is crucial in helping to maintain internal homeostasis (Sapolsky et al. 2000; Wingfield and Sapolsky 2003).

I conducted the first series of predator presentation experiments during the incubation stage, with the aim of assessing female responses to nest predation risk. I simulated nest predation risk using a decoy and recording of a common corvid nest predator, the Western scrub-jay

(*Aphelocoma californica*). I predicted that females would increase the length of incubation on-bouts to reduce the probability of nest depredation. In this case, females investing more into reproduction should display greater increases in incubation attentiveness (percentage of time spent on the nest) in response to the nest predator, despite self-maintenance costs associated with elongated incubation on-bouts (Box 3, Magnhagen 1990; Ghalambor and Martin 2000, 2001, 2002). Thus, I predicted that variation in the stress response (Box 3) and male song complexity, would correlate with female responses to nest predation risk. In addition, with respect to the nest predator presentation experiment, I also assessed plasticity in a unique type of female vocalization, the nest departure call, which may function to recruit males to guard nests during incubation off-bouts, but also entails the potential cost of attracting predators.

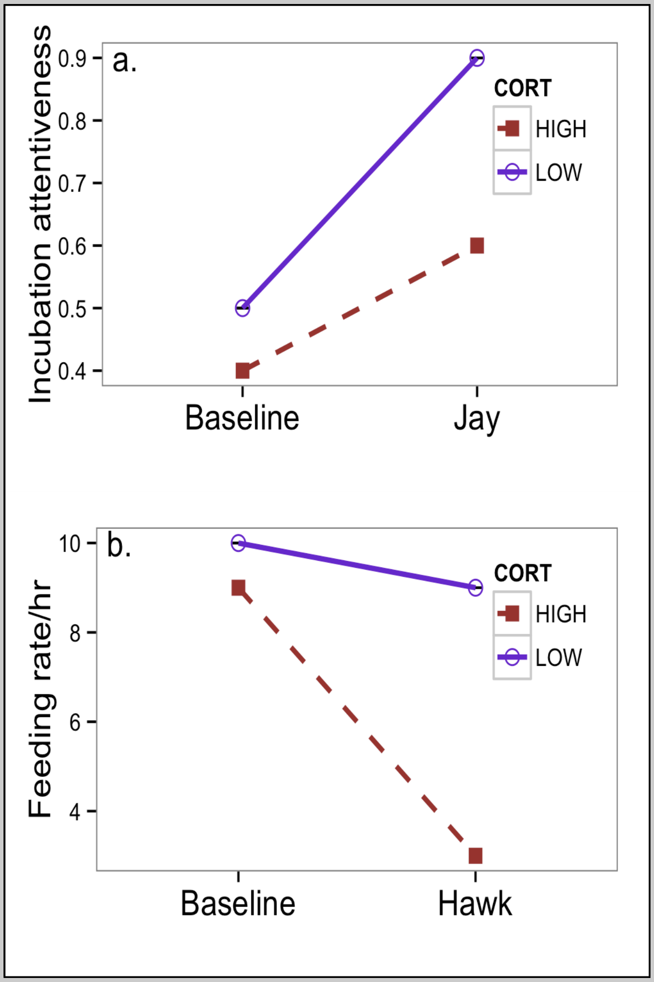
I used the second series of predator presentation experiments to assess male and female responses to adult-directed predation risk during the nestling stage, as simulated by an accipiter (sharp-shinned hawk (*Accipiter striatus*)) decoy and recording. In response to adult-directed predation risk, I predicted that individuals would decrease both nestling provisioning rates and nest attentiveness, as a means of reducing mortality risk. Further, I expected birds investing more into reproduction to display lower magnitude decreases in nestling provisioning rates in response to adult-directed predation pressure, despite the potential risk of mortality (Box 3, Magnhagen 1990; Ghalambor and Martin 2000; 2001; 2002). Thus, I predicted that variation in the stress response (Box 3) and male song complexity would correlate with responses to adult-directed predation risk in both males and females.

OVERVIEW OF DISSERTATION

This dissertation consists of six chapters. The first five chapters derive from a comprehensive study of a population of *M. melodia* breeding near the University of California's Sierra Nevada

Aquatic Research Laboratory (SNARL). In these chapters, I focus on adaptive patterns of parental effort, responses to perceived offspring- and adult-directed predation risk, and associations between male song complexity, the adrenocortical stress response, and parental behavior. In the final chapter, I examine modulation of the stress response in relation to variation in anthropogenic disturbance levels within an urban population of song sparrows breeding in Riverside, CA. I proceed to briefly outline the content of each chapter.

BOX 3. PREDICTIONS FOR PREDATOR PRESENTATION EXPERIMENTS



Predictions for individuals varying in CORT levels (high, low) with respect to incubation (a) and nestling stage (b) predator presentation experiments. I predicted that the stress response negatively correlates with parental effort and risk-taking for offspring. In (a) incubating females with low CORT display higher baseline incubation attentiveness than females with high CORT, and also a greater increase in incubation attentiveness in response to nest predation risk, reflecting a greater willing to accept self-maintenance costs to protect the brood. In (b) parents with low CORT provision nestling at higher baseline rates than parents with high CORT, and also decrease nestling provisioning rates less in response to adult-directed predation risk, reflecting a greater willingness to take risks for offspring.

Chapter 1: *Song repertoire size and song syllable diversity differentially predict the adrenocortical stress response in the song sparrow* (Melospiza melodia)

In Chapter 1, I focus on the relationship between the adrenocortical stress response and song complexity in male song sparrows. Previous research suggests that higher quality individuals with elaborate sexually selected traits may have smaller stress responses that allow intense investment into reproduction, whereas low quality individuals may have larger stress responses that promote investment into self-maintenance at the expense of reproduction and sexual display (Spencer et al. 2003; Leary et al. 2006; Almasi et al. 2008; Husak and Moore 2008; Spencer and MacDougall-Shackleton 2011). Moreover, developmental stress may concomitantly act to impact the HPA axis and sexually selected traits, including song complexity. Specifically, individuals that experience higher levels of, or are less resistant to, development stress may display larger stress responses and less elaborate sexual displays as adults (Spencer et al. 2003; Spencer and MacDougall-Shackleton 2011). Developmental stress may be particularly likely to impact song complexity in song sparrows due to a link to brain development. Further, song learning is close-ended in *M. melodia*, such that song complexity is not modified later in life (Nowicki et al. 1998; Spencer et al. 2003; Nowicki and Searcy 2004).

However, the relationship between the stress response and sexually selected traits is likely more complex. Firstly, large stress responses may confer fitness benefits in some contexts, such that sexually selected traits might positively correlate with stress hormone levels (Husak and Moore 2008). Secondly, different components of multifaceted sexually traits might convey different information about male quality, and thus correlate with the stress response in different fashions (Møller and Pomiankowski 1993; Johnstone 1996). Thus, my aim in Chapter 1 was to determine what relationship exists between the stress response and song complexity in SNARL song sparrows. Moreover, I also investigate the relationship between song complexity, the stress response, and other male quality and fitness metrics, which allows more thorough evaluation of the signaling potential of song and fitness ramifications of differences in CORT levels.

Chapter 2: *Predation risk modifies the relationship between parental effort and song complexity in the song sparrow* (Melospiza melodia)

In Chapter 2, I examine differences in paternal and maternal effort and responses to adult- and offspring-directed predation risk with respect to male song complexity. I predicted that paternal effort and responses to predation risk would vary as a function of song complexity in one of two alternative ways. Firstly, given a pattern of positive covariation between direct paternal benefits and the sexually selected trait of song (as proposed by the good parent hypothesis; Hoelzer 1989; Hill et al. 1991; Buchanan and Catchpole 2000), males with complex songs may display higher paternal effort and smaller magnitude decreases in offspring provisioning rates and nest attentiveness when confronted with adult-directed predation risk. Alternatively, given a tradeoff between mating effort and paternal effort in high quality males with complex songs (Burley 1988; Møller and Thornhill 1998; Qvarnström et al. 2000), song complexity may negatively correlate with paternal effort and positively correlate with reductions in offspring care in response to adult-directed predation risk. Further, I evaluate whether song repertoire size and syllable diversity may reflect distinct information about paternal service, as might be the case given a multiple messaging function of song (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003), and whether predation risk modifies or induces relationships between song complexity and paternal effort by elevating costs of parental care.

For females, I examine the contingency that mate quality, as reflected by song complexity, would influence parental effort and responses to nest predation risk during incubation, and adult-directed predation risk during the nestling stage in one of two alternative fashions. Specifically, the first contingency (based on the differential allocation hypothesis; Burley 1986, 1988) proposes that females increase parental effort when mated to males with complex songs, because these males provide good genes that elevate the value of the current reproductive attempt. In this case, maternal effort should positively covary with song complexity, and behavioral responses to predators should reflect a tendency to emphasize offspring wellbeing over self-maintenance. Alternatively, as proposed by the reproductive compensation hypothesis (Bluhm and Gowaty 2004; Gowaty et al. 2007; Harris and Uller 2009), females mated to males with less complex songs may increase maternal effort to ameliorate fitness declines associated with reduced offspring genetic quality or paternal support. In this case, the opposite pattern of covariation between song complex and maternal behavior might arise. Finally, I evaluate whether females may differentially allocate (or compensate) only with respect to one of the song variables, potentially suggesting a multiple messaging function of song, and whether predation risk modifies the relationship between song complexity and maternal effort.

Chapter 3: *Variation in parental effort, the adrenocortical stress response, and predation risk in the song sparrow* (Melospiza melodia)

In Chapter 3, I examine whether intraspecific variation in the stress response, as measured by plasma CORT levels, predicts variation in parental effort and risk-taking behavior. Given that down-regulation of the stress response reflects high brood value (Wingfield et al. 1995; Bókony et al. 2009), I predicted that individuals with smaller stress responses would invest more into parental care, and respond to predation risk in a fashion prioritizing brood preservation (Box 3). On the other hand, I predicted that individuals with larger stress responses would display lower levels of parental effort and respond to predators in a fashion that emphasized self-maintenance. I also investigate how baseline CORT concentrations might reflect parental effort. Specifically, empirical evidence suggests baseline CORT may either be elevated as a manifestation of poor body condition and chronic stress, in which case baseline CORT might negatively correlate with parental effort, or because individuals are investing intensely into the energetically demanding activity of breeding, in which case baseline CORT might positively correlate with parental effort (Wingfield et al. 1998; Wingfield and Sapolsky 2003; Landys et al. 2006; Romero et al. 2006; Bonier et al. 2009).

Chapter 4: *Sex-specific modulation of the adrenocortical stress response, parental effort and parental risk-taking behavior in the song sparrow* (Melospiza melodia)

In Chapter 4, I also explore the relationship between the adrenocortical stress response, parental effort, and behavioral responses to predators. However, this chapter is founded on the theory that differences in reproductive strategy based on sex should give rise to differences in modulation of the stress response over the course of the breeding season. In addition, I strove to determine whether sex-specific differences in parental effort and risk-taking are in the direction predicted by differences in CORT levels (Wingfield et al. 1995; Wingfield and Sapolsky 2003). Female birds must invest highly in ova, often are solely responsible for incubation duties, and have higher certainty of parentage, such females are expected to display lower breeding season levels of stress hormones than males (Wingfield 1984; O'Reilly and Wingfield 2001; Bókony et al. 2009). In addition, females may also display greater declines in body condition over the course of the breeding season, reflecting higher costs of reproduction (Resnick 1985; Moreno 1989; Roff 1992). However, in *M. melodia*, although only females incubate the eggs, both parents contribute to nestling provisioning. Thus, I predicted that sex differences in the stress response and body condition might be reduced during the nestling stage (Holberton and Wingfield 2003). Nevertheless, given maintenance of smaller stress responsiveness during the nestling period in females relative to males, I predicted that females would display higher parental effort and lower magnitude decreases in parental effort in response to adult-direction predation risk, despite biparental contribution to nestling care.

Chapter 5: *Phenotypic plasticity in nest departure calls: weighing costs and benefits*

In Chapter 5, I exploited the same nest predator presentation experiment described for Chapters 2 and 3. However, this chapter is distinct in that it examines patterns of adaptive plasticity in a unique, and seemingly paradoxical, behavior: the nest departure call. Incubating female song sparrows give nest departure calls when leaving the nest, and calls have acoustical properties that make them easy to locate (McDonald and Greenberg 1991). Thus, given high rates of nest depredation and parasitism, fitness costs of calling are potentially extreme, and adaptive benefits must exist that outweigh this cost. Moreover, adaptive plasticity in the production of calls is expected, such that benefits are maximized and costs limited (Yasukawa 1989; McDonald and Greenberg 1991; Clotfelter 1998). Thus, in this chapter, I explore whether females adaptively adjusted calling behavior with respect to nest predator presence, which could elevate costs of calling. Further, I explore whether females adjust calling behavior with respect to male presence and quality, which could elevate benefits of calling if calls function to promote paternal nest guarding. Finally, I examine whether nest height modifies the effect of predator presence on calling behavior, since nest height may affect the ease of nest detection by predators. Although not directly focused on parental effort, this chapter explores how plasticity in behavior based on cost-benefit analysis may facilitate reproductive success.

Chapter 6: *Variation in the adrenocortical stress response and condition metrics within a heterogeneous urban environment*

Finally, in Chapter 6, I assess variation in the stress response as a function of anthropogenic disturbance level within an urban environment. Genetic or plastic down-regulation of the acute stress response may aid individuals in coping with frequent anthropogenic disturbance, and allow for maintenance of breeding activity in an urban setting (Partecke et al. 2006; Bonier et al. 2007; French et al. 2008; Atwell et al. 2012). Further, individuals may also show changes in baseline CORT concentrations within urban environments, with elevated baseline CORT levels occurring either as a manifestation of pathological chronic stress, or as a means of coping with increased energetic demands associated with human-related habitat change (Wasser et al. 1997; Walker et al. 2005; Bonier et al. 2007; Fokidis et al. 2009; Zhang et al. 2011). The majority of previous studies on the effects of urbanization on stress physiology have evaluated broad-scale differences between one urban population and one rural population (but see Bonier 2012 for review). I evaluate differences in stress physiology on a finer scale, within an urban environment.

Specifically, I examine variation in the stress response between two distinct urban habitat types: activity centers, with high disturbance levels, and activity refuges, which are relatively buffered from disturbance. Additionally, I compare body mass, hematocrit levels, feather quality, and total antioxidant capacity between these urban habitat types, as a means of more thoroughly assessing whether song sparrows effectively cope with anthropogenic disturbance, or show evidence of pathology. I predicted that activity center birds would display suppressed acute stress responses relative to refuge birds, as a means of preventing pathological impacts on health and fitness that might result from frequent elevation of CORT. In contrast, since song sparrows are a successful urban species, I predicted that baseline CORT and other condition metrics would be comparable between urban habitat types. Although this chapter did not directly examine intraspecific variation in parental effort, modulation of the stress response in the context of human disturbance may be crucial in allowing for maintenance of reproductive effort in the face of stressors that might otherwise terminate reproductive activity and divert energy investment towards self-maintenance (Partecke et al. 2006; Atwell et al. 2012).

CONTRIBUTION OF THIS DISSERTATION

In this dissertation I demonstrate that predation risk has the potential to modify relationships between parental effort and both song complexity and the stress response. Thus, my work suggests that fitness ramifications of variation in song complexity and the stress response may be modified by predation pressure. Consequently, the strength of sexual selection, and the potential for the stress response to mediate sexual signaling, may vary as a function of the predator regime. In addition, female song sparrows also adjusted nest departure calling behavior as a function of nest predation risk, demonstrating that predation risk may also affect communication in biparental species, with potentially important ramifications for fitness.

My work also suggests that song in *M. melodia* is a multifaceted sexual signal that communicates multiple messages regarding male phenotypic or genetic quality. As a result, females may need to evaluate both dimensions of song complexity when making decisions regarding mate choice and parental investment. Indeed, predicting maternal behavior depended on knowledge of both song traits. Further, with respect to the relationship between the stress response and song complexity, I found that only syllable diversity negatively correlated with the magnitude of the stress response during adulthood. Although other explanations are possible, this result suggests that only syllable diversity may communicate developmental stress resistance, that developmental stress may affect different components of sexual ornamentation non-equivalently, and that negative correlations between the stress response and the elaboration of sexual ornaments might not always arise. Indeed, large stress responses may confer fitness benefits. Thus, one might sometimes expect high quality individuals to exhibit large stress responses.

Finally, my research on variation in the stress response within an urban environment suggests that song sparrows are an urban adapter species that successfully cope with unique selective pressure encountered in the urban realm. This work contributes to a growing suite of studies that examine effects of urbanization on stress physiology and condition, and motivates thinking regarding how pre-existing physiological control mechanisms may be modified to aid in coping with unique selective pressures encountered in anthropogenic environments.

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CHAPTER 1: Song repertoire size and song syllable diversity differentially predict the adrenocortical stress response in the song sparrow (*Melospiza melodia*)

ABSTRACT

Physiological mechanisms that pleiotropically affect condition, life-history decisions, and sexually selected traits may enforce reliable sexual signaling. The adrenocortical stress response regulates energy balance, controls the vertebrate response to survival threats, and may divert energy expenditure away from investment into costly sexual displays. Further, developmental stress may concomitantly affect the stress response and sexual signals that develop early in life, such as song in passerine birds. We examined the relationship between the stress response, as measured by corticosterone (the primary avian stress hormone) concentrations, and the sexually selected trait of song complexity in song sparrows (*Melospiza melodia*). Additionally, we explored whether the stress response or song complexity predict other male quality (body condition, wingchord, hematocrit) or fitness (survival, nestlings fledged, nestling mass) metrics. In contrast to prior research, which suggests negative relationships between sexual ornaments and the stress response, males with larger song repertoires had larger stress responses. However, males with higher song syllable diversity had smaller stress responses, suggesting that syllable diversity may reflect stress resistance. In addition, males with higher syllable diversity were larger and had lower hematocrit, males with larger song repertoires had heavier nestlings and higher hematocrit, and males with larger stress responses had higher hematocrit and lighter nestlings. Baseline corticosterone concentrations were unrelated to song complexity or quality and fitness metrics. Results suggest that the stress response may correlate differently with unique components of sexual displays, and that song repertoire size and syllable diversity may have distinct signaling functions. *Keywords*: Song complexity, adrenocortical stress response, sexual selection, multiple signaling, *Melospiza melodia*

INTRODUCTION

Theory predicts that sexually selected traits, such as song repertoire size, sexual coloration, and elaborate plumage characteristics, serve as reliable signals of male quality (Hamilton and Zuk 1982; Hill 1991; Andersson 1994). Which mechanisms enforce the honesty of these signals is a question of long-standing importance (Andersson 1994). Physiological mechanisms that have pleiotropic effects on condition, life-history decisions, and sexually selected traits may be central in translating individual quality differences into honest vocal or morphological signals (Ketterson and Nolan 1992; Husak and Moore 2008). Covariation among multiple traits may prevent low-quality males from adopting misleading phenotypes (Ketterson and Nolan 1992).

We examined one physiological mechanism that may mediate sexual signaling, the adrenocortical stress response (Husak and Moore 2008). The adrenocortical stress response is controlled by the hypothalamus-pituitary-adrenal (HPA) axis, aids in regulation of energy balance, and helps control the vertebrate response to survival threats (Saplosky et al. 2000; Romero 2002; Wingfield and Saplosky 2003). During a stress response, release of corticotropin-releasing hormone from the hypothalamus culminates in release of steroid glucocorticoids (GCs) from the adrenal cortex. Physiological baseline of circulating GCs is elevated during periods of energy demand, such as during the breeding season (Saplosky et al. 2000; Romero 2002). However, very high levels of baseline GCs may overwhelm internal coping mechanisms and lead to pathology and reduced survival probability (Romero and Wikelski 2001; Brown et al. 2005). Moreover, elevation of GCs above physiological baseline via activation of the stress response may divert energy investment away from reproduction, including production of sexually selected traits. Instead, investment in survival is promoted by mobilization of energy reserves and diversion of resources to the brain and major muscles (Astheimer et al. 1995; Wingfield and Sapolsky 2003; Hau et al 2010). Further, the stress response often suppresses the immune system (Sapolsky et al. 2000), which may inhibit expression of sexually selected traits in individuals infected by pathogens (Hamilton and Zuk 1982; Møller et al. 1998).

Thus, high quality individuals may exhibit low stress responses that reflect superior physiological condition and energy reserves, and allow for high investment into reproduction and sexual signaling. In contrast, low-quality individuals may display large stress responses that facilitate a "best of a bad job" strategy characterized by low investment into reproduction and high investment into self-maintenance (Wingfield and Saposky 2003; Husak and Moore 2008; Bókony et al. 2009; Hau et al. 2010). Indeed, low GC levels have been correlated to enhanced production of sexually selected traits (Leary et al. 2006; Husak and Moore 2008; Spencer and MacDougall-Shackleton 2011). For instance, in Great Plains toads (*Bufo cognatus*) lower baseline corticosterone (CORT, the major GC in birds, most reptiles and amphibians, and many mammals) correlates with increased song duration and female preference (Leary et al. 2006). Further, developmental stress may pleiotropically affect the HPA axis and sexually selected traits, leading to a negative correlation between the stress response and expression of sexually selected traits that persists into adulthood (Leary et al. 2006; Husak and Moore 2008; Roulin et al. 2008; Spencer and MacDougall-Shackleton 2011). Indeed, recent research in both birds and mammals has demonstrated that prolonged exposure to stress or CORT treatment during development can result in larger stress responses in adults (Pravosudov and Kitaysky 2006; Darnaudéry and Maccari 2008; MacDougall-Shackleton et al. 2009; Spencer et al. 2009).

However, the relationship between the stress response, individual quality, and reproductive investment is likely more complex than suggested above. If baseline GCs reflect average levels of stress perceived by animals in the environment, individuals of higher quality may have lower baseline GCs and may be favored as mating partners (Husak and Moore 2008). On the other hand, baseline GCs also help regulate energy balance and may be elevated during energetically demanding periods. Indeed, recent research links higher baseline GCs to reproductive success (Bonier et al. 2009; Ouyang et al. 2011; Riechert et al. 2012), and individuals that forgo breeding may have low GC levels (Goutte et al. 2010). Further, high stress-induced GCs may confer a greater capacity to cope with challenges (Breuner et al. 2008; Husak and Moore 2008). Thus, given tradeoffs between costs and benefits of elevating GCs, whether high or low HPA reactivity proves adaptive may depend on environmental conditions. For instance, in great tits (*Parus major*), individuals with small stress responses achieve higher fitness in stable environments, whereas individuals with large stress responses achieve higher fitness under unstable conditions (Cockrem and Silverin 2002). Finally, different sexually selected traits may signal distinct attributes of male genetic or phenotypic quality, as proposed by the multiple messages hypothesis (Møller and Pomiankowski 1993; Johnstone 1996; Andersson et al. 2002), such that different sexually selected traits may correlate with the stress response in different fashions.

We explored complexity in the relationship between sexually selected traits and GC levels by studying covariation between song complexity and the stress response in the song sparrow (*Melospiza melodia*). Song complexity is a multidimensional secondary sexual trait, consisting of both song repertoire size and song syllable diversity. Thus, our first objective was to establish whether both metrics of song complexity predict the magnitude of the stress response in the same fashion, with one contingency being that large stress responses are negatively associated with expression of sexually selected traits (Spencer et al. 2003; Spencer and MacDougall-Shackleton 2011). Alternatively, different song components may communicate different aspects of male quality or reproductive strategy and may thus correlate with the stress response non-equivalently

(Møller and Pomiankowski 1993; Johnstone 1996). Secondly, we were interested in the relationship between baseline CORT concentrations and song complexity, although variation in baseline CORT concentrations did not reflect song complexity in previous work on *M. melodia* (MacDougall-Shackleton et al. 2009; Schmidt et al. 2012). Baseline CORT concentrations may negatively correlate with song complexity if elevated levels reflect poor physiological condition (Romero and Wikelski 2001; Brown et al. 2005). Thirdly, we assessed whether song complexity and plasma CORT concentrations reflect other metrics of quality including body condition, wingchord, and hematocrit. Wingchord serves as a composite metric of body size and condition at molt, since larger birds have longer wingchords and birds in better condition at molt grow longer, higher quality feathers (Harper 1999). Low hematocrit may indicate reduced physiological condition associated with anemia, which can arise due to nutritional stress, dehydration, toxins, or blood loss from injury (Fair et al. 2007). In addition, hematocrit may also reflect activity level and oxygen demands, and may be higher in breeding males due to increased testosterone levels (Saino et al. 1997; Fair et al. 2007). Finally, we evaluated whether song complexity and plasma CORT levels are correlated with fitness proxies, including survival to the following breeding season, number of nestlings fledged per season, and nestling mass. Quantifying other quality and fitness metrics allowed me to more thoroughly assess the signaling function of song complexity and to more meaningfully evaluate the significance of correlations between song complexity and the stress response.

METHODS

Study species and system

In song sparrows, song learning is determinate and is completed during the first year, such that song complexity does not change during adulthood (Nordby et al. 2002; Schmidt et al. 2012).

Thus, developmental stress may act to simultaneously affect repertoire characteristics and the HPA axis (Nowicki et al. 1998; Spencer and MacDougall-Shackleton 2001). Previous research has found that male song sparrows have song repertoires sizes ranging from about 7 to16 songs. Further, song syllable diversity, or the number of distinct notes in a male's repertoire, may also contribute information about male quality (MacDougall-Shackleton et al. 2009). Song repertoire size has been positively correlated with numerous male fitness metrics, including female choice (Searcy 1984; Hiebert et al. 1989; Reid et al. 2004), male survival probability (Pfaff et al. 2007), immunocompetence (Reid et al. 2005a), territorial defense (Reid et al. 2005b), and offspring recruitment (Hiebert et al. 1989; Reid et al. 2005b).

Our study population of song sparrows breeds along Convict and McGee Creeks, on the eastern slope of the Sierra Nevada (Mono County, CA). Convict Creek flows through the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL; 37º36'51"N/118º49'47"W). McGee Creek is located ~10 miles south of SNARL in the Inyo National Forest (37°33'20"N/ 118°47'35"W). Both study sites are located at mid-elevation, between 2,100-2,500 meters.

We captured territorial males during three consecutive breeding seasons (2010-2012), between May 5 and July 27. Song sparrows in our population can fledge multiple clutches per season and repeatedly re-nest after clutch loss. Consequently, males may display cyclic patterns of hormone release. We knew the nesting status of all birds upon capture, and controlled for differences in nesting stage of capture in statistical analyses. Specifically, our entire dataset included 81 observations on 45 unique males, with early stage observations (prior to the nestling period) including 57 observations on 44 unique males and nestling stage observations including 16 observations on 15 unique males. We captured a total of 19 males repeatedly over the course of the study. Samples sizes for some statistical tests are reduced due to missing data. We banded birds with USGS aluminum bands and with an additional combination of three colored leg bands.

Field techniques were approved by the University of California, Riverside's animal care and use committee (protocol A-20100002E), and were additionally authorized by a USGS bird banding permit (23035-F), a California state collecting permit (SC-11059), a federal migratory bird collecting permit (MB22670A-0) and a special use permit from the Inyo National Forest (MLD100008P).

Measuring the adrenocortical stress response, body condition and hematocrit

We used conspecific playback to capture territorial males in mist nets between 0600 and 1600 PDT and then used standard capture protocol to measure the stress response (Wingfield et al. 1992). We took an initial blood sample within 3 minutes of capture (to characterize baseline CORT concentrations) and another sample after 30 minutes (to characterize elevated or acute CORT concentrations after stress) (Wingfield et al. 1992). We confined birds in cloth holding bags in the interlude between sampling points. We used 26-gauge needles and heparinized microcapillary tubes to withdraw small blood samples (~80 μl) from the brachial vein. We stored blood samples on ice in the field, and subsequently separated plasma from cell fraction via centrifugation for 12 minutes at 11,000 rpm. We determined hematocrit immediately following centrifugation by measuring the fraction of total blood volume occupied by erythrocytes using a Zipocrit hematocrit reader. We stored plasma at -30°C until performing CORT assays.

At the time of capture, we also measured body mass (± 0.1 g) using a digital scale, unflattened wingchord (± 1 mm) using a wing scale, and tarsus (± 0.01 mm) using digital calipers. We subsequently calculated body condition using residuals of a regression of body mass on wingchord (Albretch et al. 2005). We did not use tarsus length when calculating body mass residuals due to a non-significant correlation with body mass.

Corticosterone radioimmunoassays

We conducted corticosterone assays using a MP Biomedical I^{125} RIA kit (07-120103) previously validated for use in *M. melodia* (Newman et al. 2008). We followed kit instructions with the exception that we diluted baseline plasma samples 1:100 with steroid diluent (5 μl plasma: 495 μl diluent), and stressed samples 1:200 (5 μl plasma: 995 μl diluent). All samples were assayed in duplicate, and a control provided by the kit manufacturer was included in each assay. Intra-assay coefficients of variation averaged 10.5% and inter-assay coefficients of variation 18.1%. After determining baseline and acute CORT concentrations, we calculated the magnitude of the stress response (delta CORT) as: delta CORT = acute CORT – baseline CORT.

Characterizing song complexity

To calculate male song repertoire size and syllable diversity, we recorded song using Canon 800 series camcorders that were simultaneously used to record nesting behavior. This method provides an efficient way to obtain audio files containing long strings of male vocalizations (up to 124 minutes of total recording time). We extracted audio files from mini DV tapes and visualized .wav files via sonograms created in the acoustical analysis program *Raven Pro* (Cornell Laboratory of Ornithology). To sample repertoires, we viewed at least 300 consecutive songs or 450 total songs (Pfaff et al. 2007; MacDougall-Shackleton et al. 2009) for 93% (42 / 45) of sampled individuals. However, since all song repertoires proved to be entirely sampled after at least 150 songs we incorporated three males for which we had recorded 174, 176, and 188 songs. Number of songs counted was not related to song repertoire size (LM: $F_{1,45} = 0.44$, $R^2 = 0.01$, $\beta = 0.002 \pm 0.003$, $P = 0.51$). We determined song types via visual inspection of sonograms, with new song types being identified upon song-type switching in strings of vocalizations. After establishing song repertoire size, we determined syllable number in each song type.

Locating and monitoring nests, measuring nestling mass, and estimating survivorship

We located nests using a combination of systematic searching and behavioral observation (Martin and Geupel 1993). We then monitored nests via nest checks every 2 to 3 days until the nest was depredated or fledged. We considered a nest to have successfully fledged offspring if the nest was empty after the projected fledging date and we observed adults and offspring in the vicinity. We tabulated the total number of nestlings fledged per season for each male. In addition, as a metric of nestling condition, we weighed nestlings (± 0.1 g) on day 5 to 10 of the nestling stage. In addition, we noted whether a brown-headed cowbird (*Molothrus ater*) parasite was present in the nest to control for negative effects of parasite presence on host offspring. Over the course of three field seasons we located 166 nests, and weighed 126 nestlings from 47 nests and 29 unique males.

We estimated survival between the 2010-2011, 2011-2012, and 2012-2013 breeding seasons by noting whether or not a male returned to his breeding territory. Song sparrow males are highly philopatric, and thus can be assumed to have died over winter if not returning to the breeding grounds (MacDougall-Shackleton et al. 2009).

Statistical analysis

To incorporate multiple measurements of the stress response from individuals captured across multiple years, we used linear mixed effects (LMM) models fit with reduced maximum likelihood in R 2.15.2 (R Core Team 2012). Firstly, to assess if song complexity predicts attributes of the HPA axis, we used baseline CORT and the magnitude of the stress response (delta CORT) as dependent variables in separate models. CORT levels were square-root transformed to normalize model residuals. We entered song repertoire size and syllable diversity as fixed effects, and time, date, and stage of capture (early versus nestling) as covariates. Male identity was entered as a random effect. Males have lower delta CORT levels during the nestling stage than earlier in the season (M. Grunst, unpublished data; Bonier et al. 2009). Thus, we first ran models in the entire dataset, while including stage of capture as a covariate to control for stage effects. We then repeated the analysis, while restricting the dataset to males captured early in the season, when we captured the majority of males.

Secondly, we examined whether song complexity or CORT levels (in separate models) predict male quality metrics: body condition, hematocrit, and wingchord. Body condition and hematocrit may reflect energy balance, and may thus vary depending on stage of capture. Therefore, for these variables, we first ran models in the entire dataset, but controlled for stage effects on delta CORT by taking residuals of a regression of delta CORT with nesting stage (early versus nestling). We then repeated the analyses while restricting the dataset to early stage captures, as above. We entered stage, date, and time of capture as covariates in the analysis using the entire dataset, and date and time of capture as covariates in the analysis restricted to the early stage. Male identity was entered as a random effect. When predicting wingchord, we eliminated duplicate observations on the same male, included only early stage values of CORT, and employed a linear model.

Thirdly, we examined whether song complexity or CORT levels (in separate models) predict male fitness metrics: nestling mass, nestling number, and survival to the subsequent breeding season. For models predicting nestling mass, we applied a cube root transformation and entered the mass of each nestling separately. Further, we included nestling age, the presence of a brown-headed cowbird nest parasite, and brood size as fixed effect covariates. For the number of nestlings fledged per season, we specified a Poisson distribution to account for the distribution of count data. Finally, for models predicting survival to the subsequent breeding season, we used a general linear mixed effects model with a binomial distribution and logit link function. For all of these models, we used birds captured in both nesting stages while controlling delta CORT for stage of capture and eliminated duplicate observations on males from the same breeding season. When males were caught in both the nestling and early stage, we used early stage values of CORT. Male identity was entered as a random effect in all models, and nest number was entered as an additional random effect in models predicting nestling mass.

We reduced models via a step-wise, backwards elimination process until all explanatory variables were significant at the $\alpha = 0.05$ level. Satterthwaite approximations (implemented by the lmerTest package in R) were employed to calculate degrees of freedom for final F tests in linear mixed models.

RESULTS

Plasma CORT levels and song complexity

Song repertoire size ranged from 5 to 12 song types with a mean ± SE of 8.87 ± 0.24 and a median of 8. Syllable diversity ranged from 25 to 43 syllable types with a mean of 33.02 ± 0.62 and a median of 34. Song repertoire size and syllable diversity were correlated in our dataset (Spearman rank correlation: $r_s = 0.50$, $N = 42$, $P < 0.001$). However, much variation in syllable number remained unexplained by repertoire size.

Male song sparrows in our population had mean baseline plasma CORT concentrations of 64.25 ± 5.79 ng/ml and mean post-stress (acute) plasma CORT concentrations of 193.45 ± 8.53 ng/ml. Acute CORT concentrations were significantly higher than baseline CORT concentrations (t-test: $t_{136} = 14.25$, $P < 0.001$). The magnitude of the stress response (delta CORT) averaged 132.10 ± 8.22 ng/ml.

Baseline CORT concentrations were unrelated to song complexity, as measured by song repertoire size and syllable diversity, in both the entire or early stage dataset (LMM: $P > 0.10$ in all cases; Appendix 1, Table 1.A1). Rather, in both datasets, baseline CORT concentrations were predicted by capture date alone, being higher earlier in the season (LMM: $F_{1,88} = 4.72$, $\beta = -0.03 \pm 0.01$, $P = 0.03$, entire dataset; $F_{1,72} = 5.09$, $\beta = -0.04 \pm 0.02$, $P = 0.03$, early stage).

In the entire dataset, males with larger song repertoires had higher delta CORT (Figure 1.1a), males with higher syllable diversity had lower delta CORT (Figure 1.1b), and delta CORT was lower during the nestling stage (Table 1.1). No other covariate predicted delta CORT (LMM: $P > 0.10$; Appendix 1, Table 1.A2).

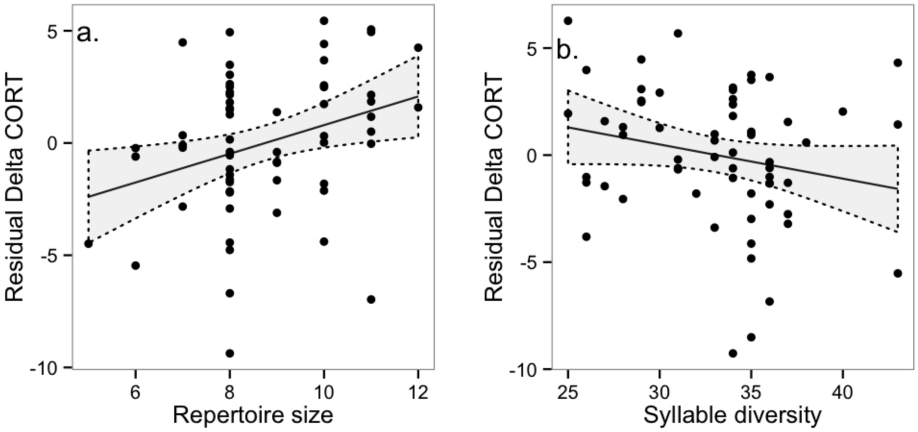

Figure 1.1. Relationships between song complexity traits and the magnitude of the stress response (delta CORT). Males with larger song repertoires had larger stress responses (a), whereas males with higher syllable diversity had smaller stress responses (b). Residual delta CORT is controlled for the effects of capture stage and the other song complexity variable. Shaded regions show 95% confidence intervals.

Males with larger song repertoires also had higher acute CORT (LMM: $F_{1,42} = 4.24$, $\beta = 0.59 \pm 0.29$, $P = 0.05$), males with higher syllable diversity tended to have lower acute CORT (LMM: $F_{1,37} = 3.00$, $\beta = -0.19 \pm 0.11$, $P = 0.09$), and acute CORT was lower during the nestling stage (LMM: $F_{1,49} = 7.93$, $\beta = -2.26 \pm 0.80$, $P = 0.01$).

Table 1.1. Linear mixed effects model predicting the stress response (delta CORT) from song complexity traits with capture stage as a covariate

	Estimate (β± SE)	F	Denom (df)	P (>F)
Repertoire size	0.83 ± 0.32	6.65	45.16	0.01
Syllable number	-0.24 ± 0.12	4.32	31.67	0.04
Stage (Nestling)	-2.17 ± 1.04	4.32	56.85	0.04

N=62 observations, 41 males.

In the analysis restricted to early stage captures, males with larger song repertoires had higher delta CORT (LMM: $F_{1,39} = 8.28$, $\beta = 1.01 \pm 0.35$, $P = 0.01$) and males with higher syllable diversity tended to have lower delta CORT (LMM: $F_{1,36} = 3.43$, $\beta = -0.25 \pm 0.14$, $P = 0.07$). No other variable predicted delta CORT within early stage males (LMM: $P > 0.10$; Appendix 1, Table 1.A2). Acute CORT did not differ as a function of song repertoire size or syllable diversity within early stage males (LMM: $F_{1,39} = 2.87$, $\beta = 0.54 \pm 0.32$, $P = 0.10$; $F_{1,38} = 1.20$, $\beta = -0.14 \pm 0.13$, $P = 0.28$, respectively), and there was no effect of capture date or time (LMM: $F_{1,39} = 1.86$, $\beta = -0.03 \pm 0.02$, $P = 0.18$; $F_{1,37} = 0.005$, $\beta = -0.01 \pm 0.16$, $P = 0.95$, respectively).

Male quality metrics with respect to song complexity and plasma CORT concentrations

Males differing in plasma CORT levels and song complexity did not differ in body condition in the entire dataset or in the analysis restricted to the early stage (LMM: $P > 0.10$ in all cases; Appendix 1, Table 1.A3 and 1.A4). The only significant predictor of body condition was stage of capture, with condition being lower during the nestling stage (LMM: $F_{1,35} = 4.74$, $\beta = -0.52 \pm 0.24$, $P = 0.04$).

However, in both datasets males with higher syllable diversity had lower hematocrit (LMM: $F_{1,68} = 10.48$, $\beta = -0.30 \pm 0.09$, $P = 0.002$, entire dataset; $F_{1,36} = 6.89$, $\beta = -0.30 \pm 0.11$, $P = 0.01$, early stage; Figure 1.2b). Further, males with larger song repertoires had higher hematocrit in the

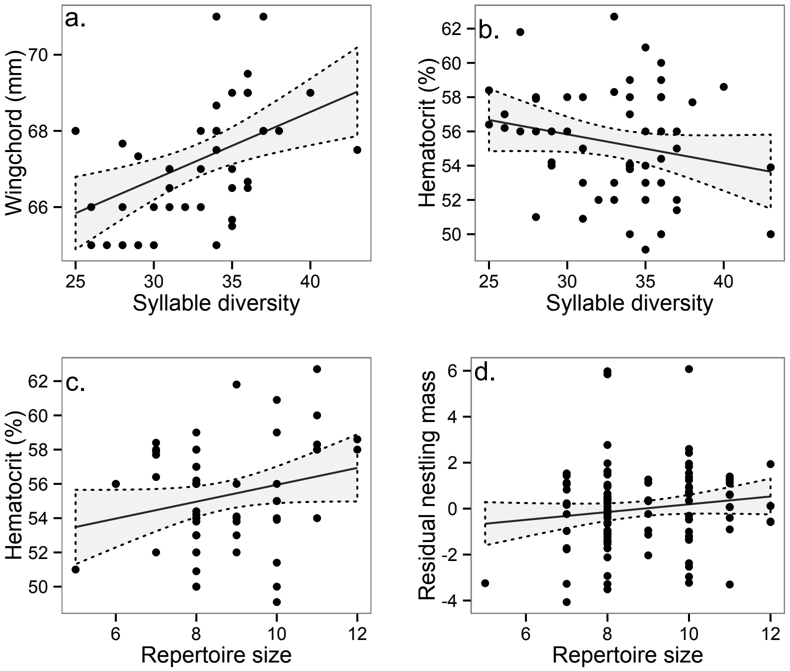

Figure 1.2. Relationships between male quality and fitness metrics and song complexity. Males with higher syllable diversity had longer wingchords (a) and lower hematocrit (b), whereas males with larger song repertoires had higher hematocrit (c) and heavier nestlings (d). Residual nestling mass is controlled for nestling age and presence of a brown-headed cowbird in the nest (b). Shaded regions represent 95% confidence intervals.

analysis restricted to early stage males (LMM: $F_{1,43} = 8.38$, $\beta = 0.85 \pm 0.30$, $P = 0.01$; Figure 1.2c) and tended to have higher hematocrit in the entire dataset (LMM: $F_{1,68} = 3.25$, $\beta = 0.47 \pm 0.26$, $P = 0.07$). Additionally, in both analyses, males with higher delta CORT had higher hematocrit (LMM: $F_{1,67} = 9.75$, $\beta = 0.29 \pm 0.10$, $P = 0.003$, entire dataset; $F_{1,65} = 6.39$, $\beta = 0.01 \pm 0.004$, $P = 0.01$, early stage). Baseline CORT also tended to be positively correlated with hematocrit, but this effect became insignificant in reduced models (LMM: $P > 0.05$; Appendix 1, Table 1.A6). Date, time and stage of capture were not related to hematocrit levels (LMM: $P > 0.10$, Appendix 1, Table 1.A5 and 1.A6).

Finally, males with higher syllable diversity had longer wingchords (LM: $F_{1,34} = 17.41$, $\beta =$

0.23 ± 0.06, $R^2 = 0.32$, $P < 0.001$; Figure 1.2a). No other variable predicted wingchord (LM: $P > 0.10$; Appendix 1, Table 1.A7).

Male fitness metrics with respect to song complexity and plasma CORT concentrations

Number of nestlings fledged per season was not predicted by any variable (LMM: $P > 0.10$; Appendix 1, Table 1.A8). In contrast, males with larger song repertoire sizes had heavier nestlings (Table 1.2; Figure 1.2d). Additionally, nestling mass increased with nestling age and decreased if a brown-headed cowbird nestling was present in the nest (Table 1.2). Perplexingly, males with higher delta CORT also had lighter nestlings despite the positive association between repertoire size and delta CORT (Table 1.2). To clarify this result, we tested whether the relationship between delta CORT and nestling mass depended on repertoire size by testing for an interaction between repertoire size and delta CORT in predicting nestling mass. Indeed, delta CORT was negatively related to nestling mass only among males with small repertoires, as indicated by a positive interaction between delta CORT (LMM, main effect: $F_{1,55} = 4.92$, $\beta = -0.01 \pm 0.005$, $P = 0.03$) and repertoire size (LMM, main effect: $F_{1,25} = 2.29$, $\beta = 0.03 \pm 0.02$, $P = 0.14$) in predicting nestling mass (LMM: $F_{1,44} = 4.34$, $\beta = 0.01 \pm 0.004$, $P = 0.04$), when controlling for the effect of nestling age (LMM: $F_{1,74} = 30.64$, $\beta = 0.05 \pm 0.01$, $P < 0.001$). Syllable diversity, baseline CORT, and brood size were not correlated with nestling mass (LMM: $P > 0.10$; Appendix 1, Table 1.A9). Finally, survival to the following breeding season was not predicted by any CORT or song complexity variable (LMM: $P > 0.10$, in all cases; Appendix 1, Table 1.A10).

Table 1.2. Linear mixed effects models predicting nestling mass from repertoire size and delta CORT when controlling for nestling age and the presence of a brown-headed cowbird (BHCO) nest parasite

	Estimate (β± SE)	F	Denom (df)	P (>F)
Repertoire size				
Repertoire size	0.03 ± 0.01	5.51	30.53	0.03
Nestling age	0.06 ± 0.01	36.48	47.80	<0.001
BHCO present	-0.08 ± 0.03	5.82	42.72	0.02
Delta CORT				
Delta CORT	-0.01 ± 0.005	4.04	50.96	0.05
Nestling age	0.05 ± 0.01	33.00	79.11	<0.001
BHCO present	-0.09 ± 0.04	5.54	52.05	0.02

N = 112 observations (nestlings), 47 nests, 29 males (Repertoire size); N = 89 observations, 41 nests, 28 males (delta CORT).

DISCUSSION

Consistent with previous studies, this study shows that characteristics of the stress response covary with sexually selected traits, thus suggesting a proximate mechanism that may modulate the honesty of sexual signals (Spencer et al. 2003; Leary et al. 2006; Roulin et al. 2008; MacDougall-Shackleton et al. 2009; Schmidt et al. 2012). Also consistent with previous work in *M. melodia*, (MacDougall-Shackleton et al. 2009; Schmidt et al. 2012) we found that the magnitude of the stress response, rather than baseline CORT concentrations, is the phenotypic component of stress physiology associated with aspects of song complexity. Unlike the magnitude of the stress response, baseline CORT concentrations did not vary with song complexity. Rather, baseline CORT varied across the breeding season with energetic demands regardless of song characteristics. Specifically, baseline CORT concentrations were high early in the season, perhaps reflecting demands of territory establishment, or low early season temperatures (Romero 2002; Landys et al. 2006). Song learning is close-ended in *M. melodia*. Thus, developmental stress may act simultaneously to affect brain regions controlling song production and the magnitude of the stress response (MacDonald et al. 2006; Spencer and MacDougall-Shackleton 2011; Schmidt et al. 2012). Moreover, since only the magnitude of the stress response covaried with song complexity, behavioral differences that also covary with song complexity may be mediated via low-affinity glucocorticoid receptors that bind CORT only at elevated plasma concentrations, rather than via high-affinity mineralocorticoid receptors (Breuner and Orchinik 2001, 2002).

However, in contrast to previous work, we found that the magnitude of the stress response negatively correlated with one metric of song complexity, syllable diversity (although this effect was marginally insignificant within early stage males alone), but positively correlated with another metric of song complexity, song repertoire size. This finding contradicts theory predicting that GCs divert energy investment towards essential growth and maintenance activities and away from secondary development of costly ornaments, and thus should negatively correlate with sexually selected signals (Husak and Moore 2008; Spencer and MacDougall-Shackleton 2011). Instead, developmental stress may act differentially on song repertoire size and syllable diversity, such that these two dimensions of song complexity serve different signaling functions (Møller and Pomiankowski 1993; Johnstone 1996). Specifically, developmental stress may act more strongly on syllable diversity, such that high syllable diversity communicates low exposure or high resistance to developmental stress, whereas song repertoire size communicates some other attribute of male genetic or phenotypic quality. Alternatively, relationships between the stress response and song complexity traits may arise during adulthood, perhaps due to males with distinct song characteristics adopting different reproductive strategies (Wingfield and Sapolsky 2003). In this case, song traits may still communicate multiple messages, such that females may need to evaluate both levels of song complexity when making decisions regarding mate choice and reproductive investment (Møller and Pomiankowski 1993).

In support of the idea that song repertoire size and syllable diversity may signal distinct messages regarding male quality, the two song traits also correlated differently with other male quality metrics. Firstly, males with higher syllable diversity had lower hematocrit, and males with larger song repertoire sizes had higher hematocrit. Lower hematocrit in males with high syllable diversity could reflect poor physiological condition (Fair et al. 2007), but this interpretation is questionable since body condition did not correlate with either syllable diversity or hematocrit levels in our dataset. Thus, lower hematocrit levels may indicate that males with lower syllable diversity are under less demand for oxygen supply, either because they employ a different reproductive strategy or because they are less challenged by the demands of breeding (Fair et al. 2007). On the other hand, the positive association between repertoire size, delta CORT and hematocrit may reflect higher activity levels (Saino et al. 1997; Fair et al. 2007). Secondly, males with higher syllable diversity also had longer wingchords, which may indicate larger size attained due to higher condition during development, developmental stress resistance, or higher condition during molt (Searcy et al. 2004; Spencer and MacDougall-Shackleton 2011). Indeed, Searcy et al. (2004) found that early-life stress had a negative effect on body size in *M. melodia*, and MacDonald et al. (2006) found that food restriction during development reduces the volume of the HVC (not an acronym), a brain region involved in song production. Therefore, developmental stress may affect size and syllable diversity concomitantly.

Further, song complexity traits also related differently to male fitness metrics. Specifically, we found that males with larger song repertoires had heavier nestlings, while syllable diversity was unrelated to nestling number or mass. Significantly, in our population, song repertoire size also positively predicts nestling stage nest attentiveness (M. Grunst, unpublished data). Taken together, these results suggest that song repertoire size may communicate paternal benefits.

We also found that males with higher delta CORT had lighter nestlings, but this negative relationship occurred only in males with small repertoires. Delta CORT may be negatively associated with nestling mass if high stress responses suppress paternal effort, as suggested by some theory (Wingfield et al. 1995; Wingfield and Sapolsky 2003). However, males with large song repertoires may resist deleterious effects of the stress response on paternal care, perhaps via modulation of down-stream mechanisms such as corticosterone-binding capacity, receptor numbers, or the efficiency of negative feedback (Breuner and Orchinik 2001, 2002; Schmitd et al. 2012).

We did not detect an effect of either the stress response or song complexity on survival between subsequent breeding seasons. This result contrasts with previous work in both the song sparrow and other species that associates elevated stress responses or baseline CORT concentrations with increased mortality rates (Romero and Wikelski 2001; Brown et al. 2005; MacDougall-Shackleton et al. 2009), and suggests that individuals with more complex songs survive longer than other individuals (Reid et al. 2005b). However, longer term monitoring of individuals might be required to detect significant relationships between song traits, the HPA axis and survival, especially in populations where extrinsic factors play a significant part in determining survivorship.

In conclusion, this work provides an important addition to a suite of recent studies that relate GCs at either baseline or elevated levels to sexually selected traits (Spencer et al. 2003; Roulin et al. 2008; MacDougall-Shackleton et al. 2009; Schmidt et al. 2012). Whereas most studies have reported negative correlations between GCs and sexually selected traits (Husak and Moore 2008), we found that two dimensions of a sexually selected trait, song complexity, correlated differently with the stress response and other male quality and fitness metrics. This result suggests that only certain attributes of a sexually selected trait may be subject to the actions of developmental stress (although patterns of covariation observed may also arise post-development), whereas other sexually selected traits may communicate different messages regarding male phenotypic or genetic quality (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994; Johnstone

1996). Indeed, large stress responses may confer fitness benefits. Thus, one might sometimes expect high quality individuals to display large stress responses.

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CHAPTER 2: Predation risk modifies the relationship between parental effort and song complexity in the song sparrow (*Melospiza melodia*)

ABSTRACT

Contrasting hypotheses exist regarding how sexual ornaments may be associated with differences in parental effort. Specifically, males with elaborate ornaments may provide more (good parent hypothesis) or fewer (tradeoff hypothesis) paternal services, and females may either increase parental effort when mated to males with elaborate ornaments (differential allocation), or increase effort when mated to less ornamented males (reproductive compensation). An unexplored contingency is that predation risk may alter relationships between ornaments and parental effort by elevating costs of parental care. We investigated the relationship between male song complexity and parental effort under baseline conditions and elevated perceived offspring- and adult-directed predation risk in song sparrows (*Melospiza melodia*). Song complexity is a multifaceted sexually selected trait consisting of both song repertoire size and syllable diversity. Intriguingly, the two song traits correlated differently with paternal effort, and predation risk altered relationships between parental effort and song characteristics. Males with larger song repertoires displayed higher nest attentiveness, and males with higher syllable diversity tended to show lower nest attentiveness, regardless of the presence of the predator. However, song repertoire size tended to be positively associated with paternal offspring provisioning rate only under adult-directed predation risk. Moreover, maternal offspring provisioning rate and the percentage of feeding visits performed by the female were associated with male song traits in a fashion that depended on adult-directed predation risk. Results suggest that song traits communicate distinct information about paternal benefits, and that females may evaluate multiple attributes of sexual displays, and environmental risk, when making parental allocation decisions.

Keywords: Parental effort, risk-taking, song complexity, good parent hypothesis, differential allocation, multiple messaging, *Melospiza melodia*

INTRODUCTION

Sexual signals, such as song complexity in birds, may reflect male quality in the form of good genes, direct benefits, or both, and may be used by females to assess brood value (Searcy 1984, 1986; Hill 1991; Kirkpatrick and Ryan 1991; Anderson 1994; Møller 1994; Searcy and Yasukawa 1996; Nowicki and Searcy 2004). Thus, variation in the expression of sexually selected traits among males within populations may predict how a male or his mate balance the survival-reproduction tradeoff (Williams 1966), and respond to offspring- and adult-directed predation risk (Ghalambor and Martin 2000, 2001). However, the nature of the relationship between sexual ornamentation and male paternal effort is the subject of debate (Kokko 1998), and alternative hypotheses have also been proposed to explain how the parental effort of females may vary with respect to male sexual displays (Harris and Uller 2009; Ratikainen and Kokko 2010). As a consequence, predicting relationships between male sexually selected traits and parental behavior is a non-trivial endeavor.

Firstly, two alternative hypotheses have been proposed to predict how elaboration of sexual displays may correlate with male paternal effort. On one hand, the good parent hypothesis (Heywood 1989; Hoelzer 1989) proposes that males with good genes better bear costs of both ornament expression and paternal effort, and that females choose males with elaborate ornaments, in part, to receive direct benefits (Hoelzer 1989; Hill 1991; Price et al. 1993; Kokko 1998). Indeed, numerous studies have shown that sexually selected traits, such as song and plumage coloration, directly relate to paternal provisioning rates or nest-guarding behavior (Greig-Smith 1982; Hill 1991; Palokangas et al. 1994; Møller and Thornhill 1998; Buchanan and Catchpole

2000; Voltura et al. 2002; Dolby et al. 2005; Halupka and Borowiec 2006). On the other hand, other studies have reported that sexual ornamentation does not reflect paternal effort (Norris 1990; Lozano and Lemon 1996; Mountjoy and Lemon 1997; Rinden et al. 2000; Smiseth et al. 2001; Hofstad et al. 2002; Darolova et al. 2012; Woodgate et al. 2012), or have reported a negative correlation between ornamentation and paternal effort (Burley 1988; Møller and Thornhill 1998; Qvarnström et al. 2000; Mitchell et al. 2007). A negative correlation between expression of sexually selected traits and paternal effort might arise if individuals preferred as mating partners invest in sexual display and pursuit of extra-pair copulations at the expense of paternal care, as proposed by the tradeoff hypothesis (Magrath and Komdeur 2003), such that benefits gained by mating with preferred partners are primarily indirect (Burley 1986; Møller and Thornhill 1998).

Secondly, two hypotheses have also been proposed to explain how females might adjust parental effort to reflect the elaboration of the sexual ornaments of their mate. On one hand, the differential allocation hypothesis (Burley 1986, 1988) proposes that females paired with "attractive" males with elaborate sexual displays gain fitness returns by investing more into parental effort, because offspring of higher genetic quality may result (Burley 1986, 1988; Harris and Uller 2009). Alternatively, the reproductive compensation hypothesis (Bluhm and Gowaty 2004; Gowaty et al. 2007; Gowaty 2008; Harris and Uller 2009) proposes that females paired to poor-quality males increase parental effort to counteract negative impacts on offspring fitness. Harris and Uller (2009) proposed that differential allocation should be more common than reproductive compensation in nature, and this conclusion was further corroborated by a recent meta-analysis in birds (Horváthová et al. 2012). However, empirical studies support both hypotheses (De Lope and Møller 1993; Saino et al. 2002; Limbourg et al. 2004; Johnsen et al. 2005; Bolund et al. 2009; Ratikainen and Kokko 2010 for review; Giraudeau et al. 2010; Pryke and Griffith 2010; Alonzo-Alvarez et al. 2012; Mahr et al. 2012; Limbourg et al. 2013; Garcia-Fernandez et al. 2013; Soma and Okanoya 2013).

An unexplored contingency is that predation risk, or other environmental or social factors that alter the costs of parental care relative to the fitness benefits of investing in offspring may induce or reduce relationships between ornaments and parental effort by accentuating the tradeoff between survival and reproduction (but see Harris and Uller 2009 for a recent model). Indeed, variation in the costs of parental care may help to explain variation in empirical relationships reported between the elaboration of sexually selected traits and paternal and maternal effort. Thus, the objective of our study was to simultaneously explore alternative hypotheses for the relationship between sexual ornamentation and both male and female parental effort in the song sparrow (*Melospiza melodia*), and to assess the contingency that increased costs of parental care associated with predation risk alter correlations between parental behavior and sexually selected traits. To this end, we determined male song complexity, a multifaceted sexually selected trait consisting of both song repertoire size (number of distinct song types) and song syllable diversity (number of distinct syllable types across song types). We then assessed parental effort in the presence and absence of perceived elevated predation risk via two presentation experiments. First, during the incubation stage, we experimentally elevated perceived nest predator risk to assess whether elevated self-maintenance costs (associated with increasing incubation on-bouts to prevent nest predation) would alter relationships between male song characteristics and maternal effort, as measured by incubation attentiveness (percent time spent incubating eggs). Second, during the nestling stage, we experimentally elevated adult-directed predation risk to assess whether imposing a direct threat to adult survival would alter relationships between song traits and both paternal and maternal effort, as measured by offspring provisioning rates and nestling stage nest attentiveness.

We evaluated specific predictions deriving from the hypotheses discussed above with respect to outcomes of predator presentation experiments. For males, the good parent hypothesis predicts that males with more complex songs display higher baseline parental effort and may maintain higher offspring provisioning rates under adult-directed predation risk, potentially inducing a relationship between paternal effort and male song traits (Figure 2.1a). The opposite pattern could if males with complex songs tradeoff mating effort over paternal care to a greater extent under predation pressure than under baseline conditions, and thus reduce provisioning effort more than other males in response to predation risk. In this case, a negative correlation between song complexity and paternal effort might exist only under predation risk (Figure 2.1b). However, in the case of the tradeoff hypothesis, we also recognized the contingency that high quality males with complex songs might provision nestlings at such low rates in the absence of predation risk, that predation pressure might act to equalize provisioning effort between males as a result of larger decreases in provisioning rates on the part of males with less complex songs. In this case, a negative correlation between song complexity and paternal effort might be more pronounced under baseline conditions (Figure 2.1c).

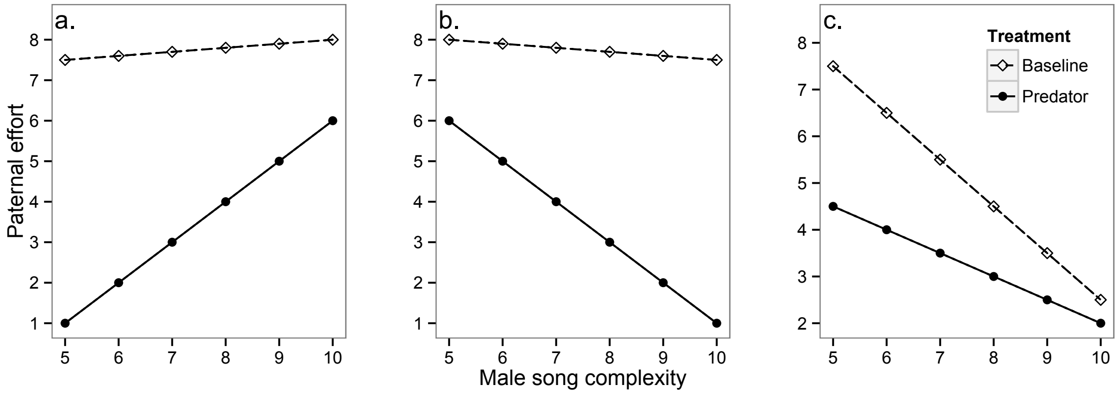

Figure 2.1. Predictions regarding how experimental elevation of perceived adult-directed predation risk might affect the relationship between male song complexity and paternal effort based on the good parent hypothesis (a), and two manifestations of the tradeoff hypothesis (b and c).

For females, the differential allocation hypothesis predicts that, relative to other females, females mated to males with more complex songs display higher baseline incubation attentiveness. Further, these females may increase incubation attentiveness more in response to nest predation risk, such that incubation attentiveness might differ as a function of song traits under predation risk, but not under baseline conditions (Figure 2.2a). In the context of adult-directed predation risk, the differential allocation hypothesis predicts that females mated with males with complex songs display higher offspring provisioning rates and nestling stage nest attentiveness. Moreover, these females may maintain higher maternal effort under adult-directed predation risk, such that song traits and female provisioning effort are only correlated in the presence of the predator (Figure 2.2b).

Conversely, a recent model by Harris and Uller (2009) suggests that reproductive compensation may only be observed when the increases in maternal effort have relatively low effects on offspring-fitness. Thus, given operation of the reproductive compensation hypothesis we predicted a larger correlation between maternal effort and male song complexity in the absence, rather than the presence, of elevated nest-directed predation risk, which poses a direct threat to offspring survival. This pattern would be achieved by females mated to males with less complex songs displaying higher incubation attentiveness in the absence of predation risk, but increasing incubation attentiveness less given a threat to the nest (Figure 2.2c). When considering offspring provisioning behavior, we also predicted that reproductive compensation would be more likely under baseline conditions than in the presence of elevated adult-directed predation risk. We made this prediction because the benefit of increasing investment to elevate the fitness of lower quality offspring would be reduced relative to survival costs given a direct threat to adult survival. Thus, given operation of the reproductive compensation hypothesis, we predicted that females mated to males with less complex songs would displaying higher offspring provisioning rates under baseline conditions, but decreasing provisioning rates more when faced with adult-directed predation risk (Figure 2.2d).

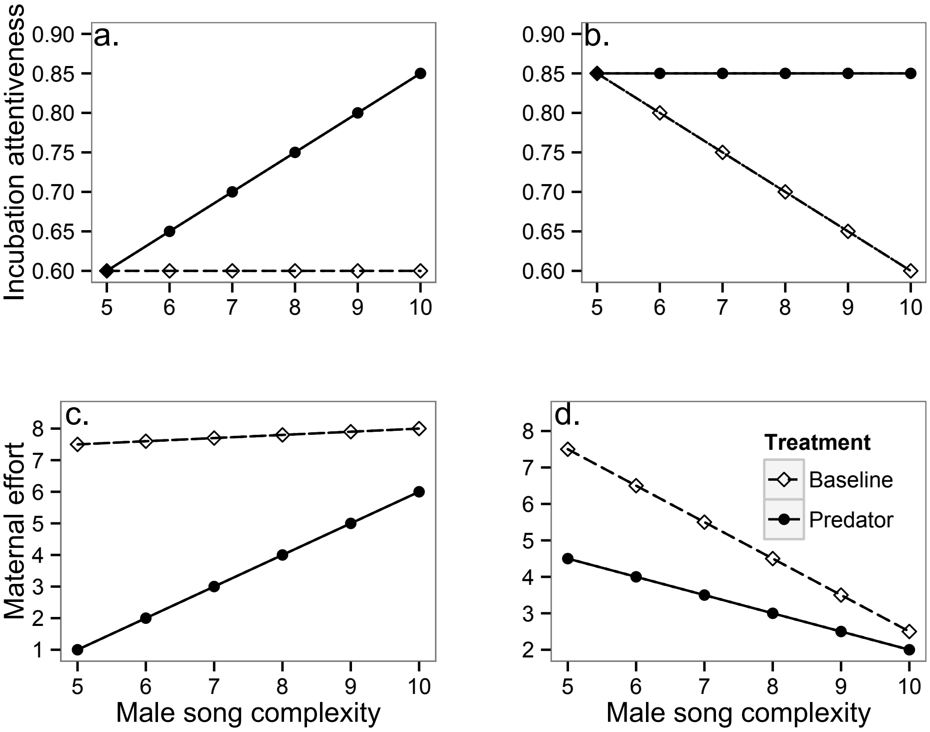

Figure 2.2. Predictions regarding how experimental elevation of perceived nest- (a, b) and adult-directed (c, d) predation risk might affect the relationship between male song complexity and maternal effort based on the differential allocation hypothesis (a, c) and the reproductive compensation hypothesis (b, d).

METHODS

Study species and site

Our study population of song sparrow breeds along the riparian corridors of Convict and McGee creeks, on the eastern slope of the Sierra Nevada (Mono County, CA). Convict Creek flows through the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL; 37º36'51"N / 118º49'47" W). McGee Creek is located ~10 miles south of SNARL in the Inyo National Forest (37°33'20"N / 118°47'35"W). We conducted research across three breeding seasons, from early May through early August, 2010 - 2012. *M. melodia* is socially monogamous and exhibits biparental care (Arcese et al. 2002). Females are sole incubators of a 2- to 6-egg clutch, but males engage in nest-guarding behavior. In addition, both males and females provision nestlings (Arcese et al. 2002). Song sparrows in our populations can fledge multiple clutches per season and repeatedly re-nest after clutch loss. Thus, our sample includes repeated measures on nests of the same nesting pair. Predation rates on nests are high with major predation threats including brown-headed cowbirds (*Molothrus ater*); corvids, such as Western scrub-jays (*Aphelocoma californica*), Steller's jays (*Cyanocitta stelleri*), and black-billed magpies (*Pica hudsonia*); short-tailed weasels (*Mustela erminea*), and garter snakes (*Thamnophis* sp.) (Latif et al. 2012; M. Grunst, personal observation).

In *M. melodia* only males sing, making song a highly sexually dimorphic and sexually selected trait. Male song sparrows have song repertoire sizes ranging from about 7 to 16 songs, and song repertoire size has been positively correlated with numerous male fitness metrics including female choice (Searcy 1984; Hiebert et al. 1989; Reid et al. 2004), male survival probability (Pfaff et al. 2007; MacDougall-Shackleton 2009a), immunocompetence (Reid et al. 2005a), territorial defense (Reid et al. 2005b), number of offspring and grand-offspring recruited to future generations (Hiebert et al. 1989; Reid et al. 2005b) and offspring sex ratio at fledging (Potvin et al. 2010). Further, in addition to song type number (song repertoire size), song syllable diversity, or the number of distinct notes in a male's repertoire, may also contribute information about male quality (MacDougall-Shackleton et al. 2009a,b).

Animal use procedures were approved by the University of California, Riverside's Animal Care and Use Committee (protocol A-20100002E) and authorized by a USGS bird banding permit (23035-F), a California state collecting permit (SC-11059), a federal migratory bird collecting permit (MB22670A-0) and a special use permit from the Inyo National Forest (MLD100008P).

Marking focal individuals and locating nests

We identified focal individuals (N = 45 pairs) by locating singing males early in the breeding season during the period of territory establishment. We target-netted birds using conspecific playback and marked individuals with a USGS aluminum band and an additional combination of three colored leg bands. In addition, we captured a smaller number of males, and the majority of females at nests during the nestling stage.

We located nests using a combination of systematic searching and behavioral observation (Martin and Geupel 1993). We then monitored nests via nest checks every 2 to 3 days until the nest was depredated or fledged. Over the course of three field seasons we located 166 nests, but due to high rates of nest predation we performed predator presentation experiments on only a subset of these nests (N = 97 nests, 45 unique males during incubation, and N = 45 nests, 31 unique males during the nestling stage).

Characterizing male song complexity

To calculate male song repertoire size and syllable diversity, we recorded song using Canon 800 series camcorders, simultaneously used to record nesting behavior. This method provides an efficient means of obtaining audio files containing long strings of male vocalizations (up to 121 minutes recording time). We extracted audio files from mini DV tapes and visualized .wav files via sonograms created in the acoustical analysis program *Raven Pro* (Cornell Laboratory of Ornithology). To sample repertoires, we viewed at least 300 consecutive songs or 450 total songs (Pfaff et al. 2007; MacDougall-Shackleton et al. 2009a) for the majority of individuals (42 of 45).

However, since all song repertoires proved to be entirely sampled after at least 150 songs or fewer, we incorporated three males for which we had recorded 174, 176, and 188 songs. Repertoire size was not predicted by number of songs counted (Linear model: $F_{1,45} = 0.44$, $R^2 = 0.01$, $\beta = 0.002 \pm 0.003$, $P = 0.51$). We determined song types via visual inspection of sonograms, with new song types identified upon song-type switching in strings of vocalizations. After song repertoire size was established, we determined syllable number in each song type. We did not determine syllable diversity for 3 males because of low-quality recordings that precluded definitive determination of syllable types.

Predator presentation experiments

Incubation stage: Female responses to nest predation risk

Nest predator presentations consisted of a taxidermic mount and recording of a Western scrub-jay. A house finch (*Haemorhous mexicanus*) decoy and recording served as a negative control. We manually compiled recordings of both jay and finch vocalizations to include an equal proportion of calling versus silence. We also recorded baseline incubation behavior in the absence of any decoy. Scrub-jay, finch, and baseline recordings were performed in randomly determined order for sequential blocks of 2 hours. Camcorders were placed 3 to 6 meters from nests and concealed to prevent disturbance originating from the recording alone. We placed decoys 6 to 10 meters from nests, 0.5 to 2 meters off the ground (Ghalambor and Martin 2000, 2001; Peluc et al. 2008), and projected recordings of vocalizations using a MP3 player placed on the ground below decoys. One presentation was conducted per breeding pair. Presentations took place between 0600 and 1400 PDT, and on day 2 to 8 of the incubation period, unless nests were more advanced when located. We controlled for the time and nesting stage at which recordings were taken in statistical analyses.

Nestling stage: Male and female responses to adult-directed predation risk

We experimentally elevated perceived adult-directed risk using a taxidermic mount and recording of a sharp-shinned hawk (*Accipiter striatus*). As for nest predator presentations, a house finch decoy and recording served as a negative control, and we also recorded baseline behavior in the absence of a decoy. We conducted adult-directed predation trials as described for nest predator presentations, on day 2 to 8 of the nestling period. We controlled for time and day in the nesting stage in statistical analyses.

Data extraction from video-recordings

Following the field season we viewed video-recordings to determine parental behavior under baseline conditions and in the context of predation risk. We determined incubation attentiveness (percent time female spent incubating eggs) by noting times of female arrivals and departures from the nest. Offspring provisioning rates were determined by counting number of feeding visits by males and females. Since song sparrows are monomorphic, we used colored leg bands to identify the sexes, in combination with behavioral cues such as female brooding (sitting on nestlings) and male song production. In addition, we also determined male and female nestling stage nest attentiveness (percent time spent on the nest) for nests recorded during 2012. Females spend time on the nest brooding nestlings since altricial young have poorly developed thermoregulatory capacity. Although males do not brood nestlings, some males guard the nest after delivering food to offspring (Arcese et al. 2002; personal observation).

Statistical analysis

We used linear mixed effects models (LMM) in the lme4 package of R 2.15.2 (R Core Team 2012) to analyze data. Models were fit with reduced maximum likelihood, and reduced via a step-wise reduction procedure until all terms retained were significant ($\alpha = 0.05$). We used Satterthwaite approximations (implemented by R package lmerTest) to calculate degrees of freedom for final F tests in linear mixed models.

We first ran preliminary analyses to determine if the baseline, predator (hawk/jay), and negative control (finch) treatments significantly affected parental behaviors measured. The finch treatment (negative control) was not significantly different than the baseline treatment in preliminary analyses (see results). Thus, we recoded finch recordings as baseline for use in all final models that included data from finch treatments. However, we did not have data from finch treatments for nestling stage nest attentiveness, since nestling stage nest attentiveness was determined from recordings taken in the final year of the study (2012), and we had previously determined that sparrows did not perceive the finch as a threat.

Secondly, we tested the effect of male song characteristics on male behavior. Specifically, we were interested in the effect of elevated adult-directed predation risk and male song traits on male offspring provisioning rates and male nest attentiveness during the nestling stage. Thus, we included male provisioning rate and male nest attentiveness as dependent variables in separate models, with treatment (hawk, baseline), song repertoire size, and syllable diversity as fixed effects. We included interactions between treatment (hawk, baseline) and song characteristics, and between the two song traits, because we were interested in the effect of song traits on behavioral modification under predation risk, and because song characteristics may interact to communicate information about individual quality or behavioral strategies. We included brood size, day in the nesting stage, time, and day in the breeding season in models as covariates. Nest number and male and female identity were included as random effects.

Next, we tested for an effect of male song traits on female behavior. Specifically, we were interested in the effect of nest predator presence and male song traits on female incubation attentiveness and elevated adult-directed predation risk and male song traits on female provisioning rate and nest attentiveness during the nestling stage. We square-root transformed female provisioning rates and squared incubation attentiveness to achieve normality of model residuals. We constructed models as described for males.

Finally, we evaluated whether song traits affected the relative contributions of males and females to offspring provisioning behavior. The percentage of provisioning visits performed by the female was used as the dependent variable, with 1s and 0s removed to achieve normality of model residuals (which did not qualitatively alter model results). We constructed models as described above.

In addition, we sought to clarify the effect of predator presence on the relationship between male song complexity and offspring provisioning by re-running the models (minus the treatment term and associated interactions) within the baseline and predation treatment separately.

RESULTS

Male song characterization

Song repertoire size ranged from 5 to 12 song types with a mean ± SE of 8.87 ± 0.24 and syllable diversity ranged from 25 to 43 syllable types with a mean of 33.02 ± 0.62. Song syllable diversity and song repertoire size were positively correlated, but much variation in each song complexity variable remained unexplained by variation in the other (Spearman rank correlation: $r_s = 0.50$, $N = 42$, $P < 0.001$).

Male song complexity and male responses to adult predators

The finch (negative control) treatment was not significantly different from the baseline treatment in the model predicting male offspring provisioning rates, or any subsequent model (LMM: $P > 0.50$ in all cases; Appendix 2, Table 2.A1), so we recoded finch trials as baseline for the purpose of final analyses. Males reduced offspring provisioning rates in response to adult-directed predation risk (LMM: $F_{1,84} = 11.83$, $\beta = -0.95 \pm 0.28$, $P = 0.001$). However, male song characteristics and adult-directed predation risk did not interact to predict male provisioning rate (LMM: $P > 0.10$; Appendix 2, Table 2.A2). There was also no evidence that males differing in song characteristics provisioned offspring at higher rates across treatments (LMM: $P > 0.10$, Main effects; Appendix 2, Table 2.A2). Brood size (LMM: $F_{1,45} = 21.68$, $\beta = 1.03 \pm 0.22$, $P < 0.001$) and day in stage (LMM: $F_{1,131} = 36.18$, $\beta = 0.52 \pm 0.09$, $P < 0.001$) both had strong positive effects on provisioning rates, but time of day did not have predictive power (LMM: $P > 0.10$; Appendix 2, Table 2.A2).

Table 2.1. Linear mixed effects models predicting paternal provisioning rate (feeding trips/hr) from male song characteristics within the predator (hawk) and baseline treatments

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Within predator treatment[a]				
Repertoire size	0.43 ± 0.22	3.97	29.15	0.055
Brood size	1.05 ± 0.24	19.79	41.10	<0.001
Day in stage	0.57 ± 0.10	32.25	17.99	<0.001
Within baseline treatment[b]				
Syllable number	-0.58± 0.45	1.66	20.15	0.25
Repertoire size	0.49 ± 0.42	1.37	28.73	0.21
Brood size	1.08 ± 0.23	21.39	27.54	<0.001
Day in stage	0.65 ± 0.13	25.03	57.58	<0.001
Syllable × Repertoire	0.40 ± 0.49	0.67	25.19	0.42

[a] N = 55 observations, 45 nests, 35 females, 31 males.

[b] N = 68 observations, 43 nests, 35 females, 30 males.

Nevertheless, when we restricted analysis to the predator treatment, we found that males with large song repertoires tended to provision offspring at higher rates, when controlling for brood size and day in the nesting stage (Table 2.1). Syllable diversity was not related to male provisioning rate under predation risk (LMM: $F_{1,17} = 0.17$, $\beta = -0.19 \pm 0.46$, $P = 0.68$), and the two song traits did not interact (LMM: $F_{1,25} = 0.06$, $\beta = -0.12 \pm 0.49$, $P = 0.80$). When restricting the analysis to the baseline treatment male song complexity was not related to male provisioning behavior (Table 2.1). Male song traits and predation risk also did not interact to predict male nest attentiveness (LMM: $P > 0.10$; Appendix 2, Table 2.A3). However, independent of treatment and other covariates, males with larger song repertoires were more

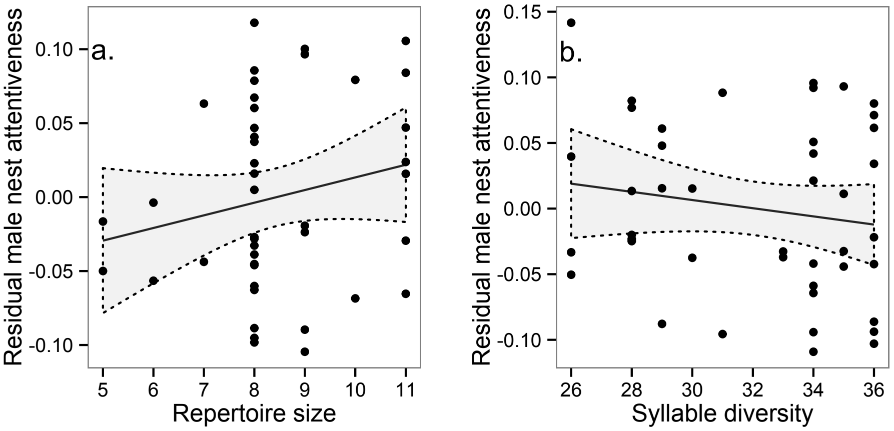

Figure 2.3. Relationships between male song traits and paternal nestling stage nest attentiveness. Males with larger song repertoires were more attentive at nestling stage nests relative to males with smaller repertoires, when controlling for syllable number. Residuals control for syllable diversity (a). In contrast, males with higher syllable diversity, when controlling for repertoire size, tended to be less attentive. Residuals control for song repertoire size (b). Shaded regions show 95% confidence intervals.

attentive at nestling stage nests (LMM: $F_{1,18} = 7.89$, $\beta = 0.04 \pm 0.02$; $P = 0.01$, Figure 2.3a). In contrast, males with a higher syllable number tended to be less attentive, although this effect was not statistically significant (LMM: $F_{1,18} = 3.31$, $\beta = -0.01 \pm 0.006$, $P = 0.08$; Figure 2.3b). No other variables predicted male nest attentiveness (LMM: $P > 0.10$; Appendix 2, Table 2.A3).

Male song complexity and female responses to nest- and adult-directed predation risk

Females reduced incubation attentiveness in response to the corvid nest predator (LMM: $F_{1, 121} = 7.30$, $\beta = -0.06 \pm 0.02$; $P = 0.01$), and incubation attentiveness decreased later in the day (LMM: $F_{1, 165} = 7.01$, $\beta = -0.01 \pm 0.005$; $P = 0.01$), and later in the incubation stage ($F_{1, 86} = 5.22$, $\beta = -0.01 \pm 0.003$, $P = 0.02$). However, male song traits were not associated with maternal incubation attentiveness either in the presence or absence of predation risk (LMM: $P > 0.10$; Appendix 2, Table 2.A4).

In contrast, male song characteristics and predator presence interacted in a complex fashion to predict maternal provisioning rates. As for males, there was an overall negative effect of predator presence on maternal provisioning rates (Table 2.2). Further, females mated to males with large song repertoires reduced offspring provisioning rates more, and thus had lower offspring provisioning rates, under adult-directed predation risk than females mated to males with smaller song repertoires (significant negative song repertoire size × treatment interaction term; Table 2.2; Figure 2.4a). The main effect of song repertoire size on maternal provisioning rate was not significant, although there was a trend towards a negative relationship (Table 2.2). Male syllable diversity had the opposite overall effect on female responses to adult-directed predation risk. When confronted with a survival threat, females mated to males with high syllable diversity reduced offspring provisioning rates less, and thus displayed higher provisioning rates under predation risk, than females mated to males with smaller syllable repertoires (significant positive syllable diversity × treatment interaction term; Table 2.2; Figure 2.4b). In addition, across treatments there was also a positive relationship between syllable diversity and maternal

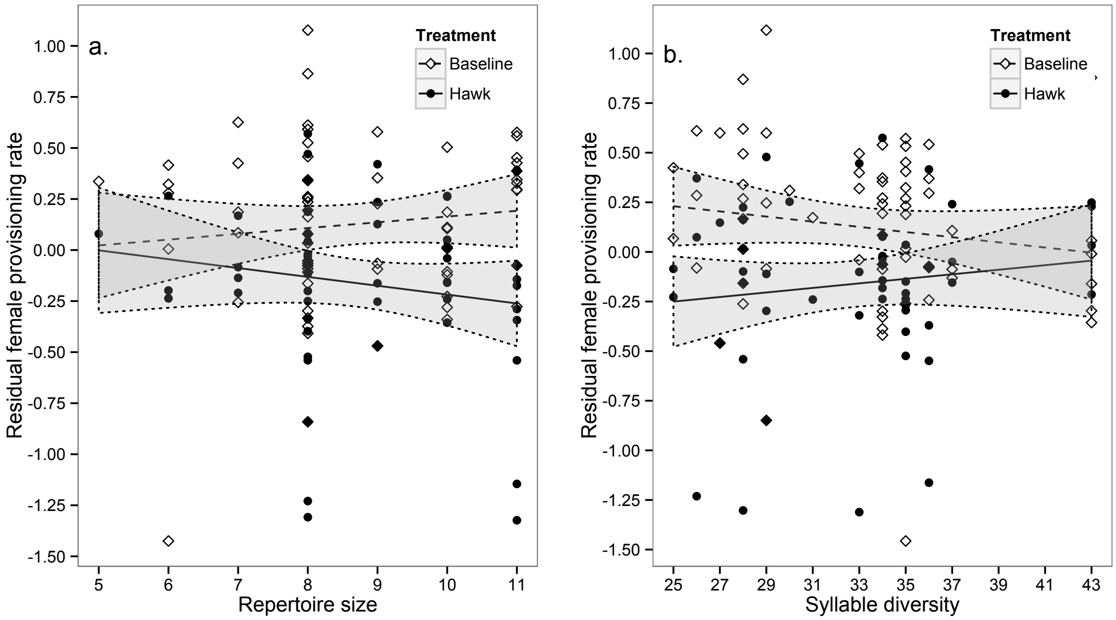

Figure 2.4. Relationships between maternal provisioning rate and male repertoire size (a) and syllable diversity (b) within the baseline treatment (dashed line, open triangle) and predation (hawk) treatment (solid line, filled circle). Females reduced provisioning rates more under predation risk when mated to males with larger repertoire size (a), but lower syllable diversity (b). Residual female provisioning rate is controlled for the effects of brood size, nestling age, and the other song complexity variable.

Table 2.2. Linear mixed effects model predicting maternal provisioning rate (feeding trips/hr) from treatment (predator presence) and male song characteristics

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Treatment	-0.29 ± 0.08[a]	12.21	74.14	<0.001
Syllable number	0.11 ± 0.10	5.96	31.99	0.02
Repertoire size	-0.03 ± 0.10	2.40	35.73	0.13
Brood size	0.19 ± 0.05	12.25	33.14	0.001
Day in stage	0.18 ± 0.03	46.89	111.90	<0.001
Treatment × Syllable	0.23 ± 0.09	5.68	74.09	0.02
Treatment × Repertoire	-0.22 ± 0.09	5.11	74.39	0.03
Syllable × Repertoire	0.27 ± 0.10	6.36	31.41	0.02

[a] Predator treatment relative to baseline.
N = 121 observations, 43 nests, 35 females, 30 males.

provisioning rate (main effect of syllable diversity; Table 2.2). Moreover, there was also a positive interaction between syllable number and repertoire size in predicting maternal provisioning rate independent of treatment (Table 2.2; Figure 2.5). This interaction reflected the fact that, across treatments, females mated to males with large song repertoires provisioned offspring at lower rates, but only if syllable diversity was also low (see gradient across bottom of Figure 2.5), whereas females mated to males with high syllable diversity provisioned offspring at higher rates, but only if repertoire size was also high (see gradient on left side of Figure 2.5). Finally, maternal feeding rate increased with brood size and day in the nesting stage (Table 2.2). Time had no effect on maternal feeding rates (LMM: $F_{1,90} = 0.002$, $\beta = -0.001 \pm 0.02$, $P = 0.96$).

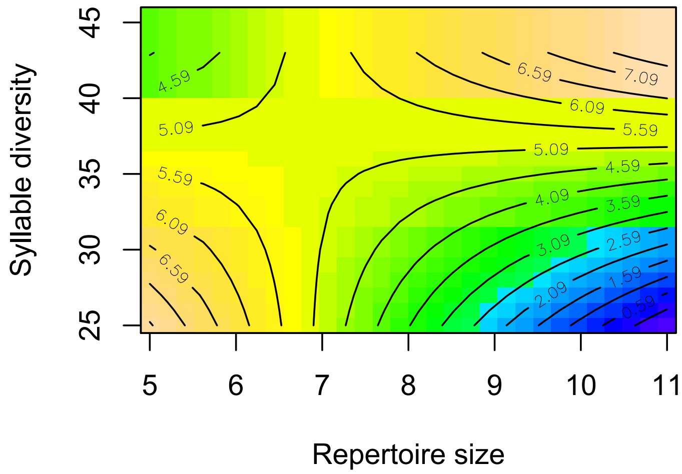

Figure 2.5. Contour plot showing the relationship between maternal provisioning rate (feeding trips/hr) and song complexity traits. Contour intervals represent 0.50 provisioning trips/hr. Warm (red-yellow) color corresponds to higher provisioning rates.

In addition, we found that within the predation treatment, females mated to males with larger song repertoires provisioned nestlings at lower rates, whereas females mated to males with higher syllable diversity provisioned nestlings at higher rates, when controlling for the positive effects of brood size and nestling age (Table 2.3). Further, there was still a positive interaction between the song traits in predicting maternal provisioning rate (Table 2.3). In contrast, within the baseline treatment the main effect of repertoire size and syllable diversity on maternal provisioning rate were insignificant, but there was still a positive interaction between repertoire size and syllable diversity in predicting maternal provisioning rate, when controlling for the effects of brood size and nestling age (Table 2.3).

Females also decreased nestling stage nest attentiveness in response to adult-directed predation risk (LMM: $F_{1,50} = 13.88$, $\beta = -0.23 \pm 0.06$, $P < 0.001$). However, maternal nestling stage nest attentiveness was not related to male song complexity or any other variable (LMM: $P > 0.10$; Appendix 2, Table 2.A5).

Table 2.3. Linear mixed effects models predicting maternal provisioning rate (feeding trips/hr) from male song characteristics within the predator (hawk) and baseline treatments

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Within predator treatment[a]				
Syllable number	0.40 ± 0.12	10.20	18.75	0.01
Repertoire size	-0.31 ± 0.12	6.58	23.20	0.02
Brood size	0.21 ± 0.07	8.40	30.10	0.01
Day in stage	0.13 ± 0.04	10.62	23.40	0.003
Syllable × Repertoire	0.27 ± 0.14	3.85	28.21	0.05
Within baseline treatment[b]				
Syllable number	0.10 ± 0.08	1.40	23.65	0.25
Repertoire size	0.008 ± 0.08	0.01	31.03	0.93
Brood size	0.20 ± 0.05	15.82	22.26	<0.001
Day in stage	0.18 ± 0.03	32.17	61.72	<0.001
Syllable × Repertoire	0.30 ± 0.09	9.52	61.72	0.01

[a] N = 53 observations, 43 nests, 35 females, 30 males.
[b] N = 68 observations, 43 nests, 35 females, 30 males.

Male song complexity and percent female provisioning under adult-directed predation risk

Adult-directed predation risk interacted with male song characteristics to predict the percentage of feeding visits performed by the female. Specifically, females mated to males with larger song repertoire size performed a lower percentage of feeding trips (LMM: Main effect; $F_{1,34} = 13.24$, $\beta = -0.03 \pm 0.02$, $P = 0.001$) and this effect depended on the presence of the predator (LMM: Treatment × repertoire size interaction term; $F_{1,64} = 19.55$, $\beta = -0.10 \pm 0.02$, $P < 0.001$). In contrast, females mated to males with higher syllable diversity performed a higher percentage of feeding visits (LMM: Main effect; $F_{1,25} = 8.17$, $\beta = 0.03 \pm 0.02$, $P = 0.01$) and this effect also depended on predator presence (LMM: Treatment × syllable diversity interaction term; $F_{1,62} = 15.30$, $\beta = 0.09 \pm 0.02$, $P < 0.001$). Predator presence did not induce an overall shift in the percent of feeding visits performed by females (LMM: $F_{1,60} = 0.07$, $\beta = 0.01 \pm 0.02$, $P = 0.79$). Further, the two song traits did not interact, and no other covariates affected percent female provisioning (LMM: $P > 0.10$; Appendix 2, Table 2.A6).

When we restricted the analysis to the predation treatment, females mated to males with larger song repertoires performed a smaller percentage of feeding visits (LMM: $F_{1,32} = 17.67$, $\beta = -0.09 \pm 0.02$, $P < 0.001$), whereas females mated to males with higher syllable diversity performed a greater percentage of total feeding visits (LMM: $F_{1,35} = 10.93$, $\beta = 0.02 \pm 0.007$, $P = 0.002$). In contrast, when we restricted the analysis to baseline recordings, male song characteristics did not predict the percentage of feeding visits performed by the female (LMM: $P > 0.10$; Appendix 2, Table 2.A6). Time of day, brood size, and nestling age all failed to predict percent female provisioning within either the predation or baseline treatment (LMM: $P > 0.10$; Appendix 2, Table 2.A6).

DISCUSSION

Rather than supporting one of the distinct hypotheses for the relationship between the elaboration of sexually selected traits and paternal and maternal effort, our study yielded the intriguing result that song repertoire size and syllable diversity were related to both paternal and maternal behaviors in different ways, supporting the multiple messages hypothesis for the information content of sexually selected traits (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003). Moreover, we found that predation risk has the potential to alter relationships between male song traits and both maternal and paternal behavior, such that correlations between some behaviors and song traits were only apparent under predation risk, as elaborated below.

Firstly, when considering paternal behavior, we found that, when controlling for syllable number, males with larger song repertoire size provided more parental support in the form of higher nestling stage nest attentiveness. Moreover, under elevated perceived adult-directed predation risk, there was a nearly significant ($P = 0.055$) positive relationship between song repertoire size and paternal offspring provisioning rates. Thus, results suggest that repertoire size may communicate information about paternal benefits, in a fashion consistent with the good parent hypothesis (Hoelzer 1989). Moreover, results for paternal offspring provisioning tend to be consistent with our prediction, illustrated in Figure 2.1a, that elevated costs of paternal care associated with predation risk may induce relationships between song complexity and paternal effort. Corroborating the idea that repertoire size may signal paternal benefits, nestling mass is positively correlated to song repertoire size (but not syllable diversity) in our population (M. Grunst, unpublished data). In contrast, when controlling for repertoire size, syllable diversity was either associated with lower nest attentiveness (there was a trend towards a negative relationship, $P = 0.08$), or was not related to nest attentiveness, and was unrelated to paternal offspring provisioning rates. Thus, results suggests that syllable diversity may either be negatively associated with paternal care, in a fashion consistent with the tradeoff hypothesis (Magrath and Komdeur 2003), or, more likely, that females do not gain information about paternal services by evaluating syllable diversity.

Past studies have also reported that one sexually selected trait, or one component of a sexually selected display, positively reflects paternal effort while another does not, or that two sexually selected traits differentially reflect paternal services (Senar et al. 2003; Dolby et al. 2005; Quesada and Senar 2007). For instance, in great tits (*Parus major*), males with large black melanin-based breast stripes defend nests more vigorously, whereas carotenoid-based plumage coloration fails to predict nest defense, but does predict body condition and nestling provisioning rates (Senar et al. 2003; Quesada and Senar 2007). Moreover, different components of a sexual signal may communicate distinct reproductive strategies. For example, in common yellowthroats (*Geothlypis trichas),* sexually selected plumage traits are correlated with within-pair mating success, whereas song rate is related to extra-pair mating success (Taff et al. 2012). To our knowledge, no previous study in *M. melodia* has examined how different song traits relate to paternal care. However, distinct patterns of correlation between song traits and other behavioral, quality and fitness metrics have been reported, which is consistent with the idea that repertoire size and syllable diversity may play different signaling roles (MacDougall-Shackleton et al. 2009b; authors, unpublished data).

When considering female behavior during the nestling stage, results also supported the multiple messages hypothesis for the signaling potential of song, and suggested that predation pressure may alter relationships between song traits and maternal effort. Specifically, when exposed to adult-directed predation risk, females mated to males with large song repertoires reduced offspring provisioning rates more than other females. Further, females mated to males with large song repertoire size also performed a lower percentage of overall feeding visits under predation risk. Thus, a pattern consistent with the reproductive compensation hypothesis was observed for the relationship between male repertoire size and maternal effort, but only under predation risk (Sheldon 2000; Gowaty et al. 2007, Gowaty 2008). Significantly, this pattern did not agree with our prediction, depicted in Figure 2.2d (contrast to result in 2.4a), that reproductive compensation might be more pronounced under baseline conditions due to lower costs of parental care relative to fitness benefits to offspring. However, our result may arise because costs of reducing maternal care are not equal across females. Females mated to males with smaller song repertoires may reduce offspring provisioning rates less under predation pressure, despite survival costs, as a means of compensating for severe fitness declines that might result from reducing provisioning of genetically inferior offspring that may be vulnerable to stress induced by food deprivation. Conversely, females mated to males with large song repertoires may be able to afford reducing provisioning rates if higher quality offspring are more stress resistant (Bluhm and Gowaty 2004; Gowaty et al. 2007; Gowaty 2008). Further, increased paternal support provided by males with large song repertoires might ameliorate costs of reduced female provisioning effort. Indeed, males with large song repertoires tended to provision offspring at higher rates under predation pressure, but not under baseline conditions. Moreover, males with larger song repertoires contributed a greater percentage of total feeding visits under predation risk. Thus, this result highlights the importance of jointly considering male and female behavior when attempting to predict the relationship between elaboration of sexually selected traits and parental effort.

Conversely, when exposed to adult-directed predation risk, females paired to males with high syllable diversity reduced feeding rates less than other females. Further, females mated to males with high syllable diversity also performed a higher percentage of overall feeding visits under predation risk. Thus, a pattern consistent with differential allocation was observed for the relationship between syllable diversity and maternal effort, but only under predation risk (Burley 1986, 1988). Further, this pattern was consistent with our prediction, depicted in Figure 2.2c (and mirrored by 2.4b), that differential allocation might emerge under predation risk due elevated costs of maternal care motivating greater decreased in care on the part of females mated to males with less complex songs. Females mated to males with higher syllable diversity may be more willing to take risks for offspring if syllable diversity reflects indirect benefits of good genes, as proposed by the differential allocation hypothesis (Burley 1986, 1988). Indeed, past research has reported that females increase solicitation behavior or reproductive investment when males demonstrate the ability to sing "sexy syllables" or physically demanding songs, composed of particular syllable types (Vallet et al. 1998; Leitner et al. 2005; Cardoso et al. 2007, 2009). Thus, it may be the presence of certain syllable types in a male's repertoire that signals quality to females, and the probability of having these syllable types may increase with syllable diversity. Moreover, in our population, males with high syllable diversity have longer wingchords and tend to have smaller adrenocortical stress responses (M. Grunst, unpublished data), which may reflect resistance to developmental stress conferred by genetic quality (Spencer et al. 2003, 2005; Husak and Moore 2008; Spencer and MacDougall-Shackleton 2011). Male syllable diversity was only weakly related to paternal effort, although there was a trend towards males with higher syllable diversity being less attentive at nestling stage nests. Therefore, it is unlikely, though possible, that variation in the paternal contribution of males differing in syllable diversity contributes to explaining differences in female parental behavior.

Intriguingly, the distinct associations between male song traits, female offspring provisioning rate, and the percentage of total feeding visits performed by the female were only apparent in the presence of the predator, and not under baseline conditions. This result suggests that elevated costs of parental care caused by elevated predation risk may induce relationships between female parental effort and male song traits, perhaps by accentuating the tradeoff between survival and current reproduction (Williams 1966). Indeed, a recent study on rock sparrows (*Petronia petronia*) found that males differentially allocate with respect to sexually selected plumage pigmentation of females when engaging in costly nest defense behavior against a weasel predator, but not when provisioning nestlings, which may entail lower self-maintenance costs (Griggio et al. 2003; Matessi et al. 2009). In contrast, Burley's original (1988) paper reported the opposite pattern, with differential allocation being apparent with respect to nestling provisioning, but not nest defense. However, as also noted by Matessi et al. (2009), Burley's nest defense behavior did not involve direct elevation of risk to the adult, and rather, entailed various forms of vigilance behavior. Significantly, given that predation risk does induce relationships between song traits and maternal effort, and that differential allocation has the potential to magnify fitness differences between males (Sheldon 2000), the strength of sexual selection for syllable diversity may increase under conditions of elevated predation pressure. On the other hand, reproductive compensation has the potential to decrease fitness differences between males (Sheldon 200), such that the strength of sexual selection for repertoire size may decrease under predation risk.

Nevertheless, in addition to independent effects of each song trait on female provisioning behavior, which emerged only under predation risk, there was also a positive interaction between song repertoire size and syllable diversity in predicting female provisioning rate, and this effect was present regardless of the presence of the predator. This interaction reflected the fact that, across treatments, syllable diversity positively predicted female feeding rate, but only when repertoire size was also high, whereas repertoire size negatively predicted female feeding rate, but only when syllable diversity was also low (see Figure 2.5). Thus, females displayed higher offspring provisioning rates when mated to males with both high values for both song complexity traits and low values of both song complexity traits. This dynamic suggests that female may differentially allocate when their mate displays high overall song complexity, but may reproductively compensate given low overall song complexity in their mate. The more complex dynamic that emerges under predation risk may arise from interactions with male behavior, since males with larger song repertoires tended to provision offspring at high rates than other males only under predation pressure.

As a caveat, we recognize that these results are correlational. Thus, we cannot conclusively determine whether patterns of maternal behavior observed are a direct consequence of assessment of male song traits, a reaction to male behavior, or the outcome of some other correlated factor such as territory or female quality (Sheldon 2000). Indeed, males with certain song characteristics may be able to secure higher-quality territories, which may affect optimal patterns of female behavior (Yasukawa et al. 1980). Similarly, males with certain song characteristics may secure mates of higher quality, such that variation in female behavior may reflect differences between females rather than female assessment of, and reaction to, male traits (Sheldon 2000).

Further, maternal effort related to male song traits only during the nestling stage, in the face of adult-directed predation risk, and not during incubation, when a threat to the brood was entailed. Correlations between maternal effort and male song traits might have arisen only during the nestling stage since nestlings are more costly to replace than eggs, which may elevate benefits of differential allocation decisions (Sheldon 2000), or because adult-directed predation risk entails a direct cost to adult survival, whereas nest predation risk does not. Further, females responded to nest predation risk by decreasing incubation attentiveness, contradicting our prediction that some females should increase attentiveness, despite self-maintenance costs, to decrease the probability of nest predation. Decreased incubation attentiveness might have resulted if latency to return to the nest increased as a means of preventing nest detection (Ghalambor and Martin 2001; Lima 2009). However, in this case, females would not face self-maintenance costs, and a relationship between mate quality and changes in female behavior might not be expected.

In conclusion, our study suggests that two components of song complexity are not merely redundant signals that enforce signal honesty and communicate equivalent information regarding paternal benefits. Rather, these traits displayed different relationships with paternal and maternal effort, suggesting a multiple messaging function. Moreover, we found that the correlation between male song complexity and paternal offspring provisioning rates, and distinct correlations between song traits and maternal offspring provisioning rates were only detectable under predation risk, and not under baseline conditions. We found no support for the prediction that reproductive compensation may only be present given lower fitness benefits to offspring relative to self-maintenance or survival costs. Rather, results suggest that elevated costs of parental care associated with predation risk may induce patterns consistent with either differential allocation or reproductive compensation, potentially altering dynamics of sexual selection.

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CHAPTER 3: Variation in parental effort, the adrenocortical stress response, and predation risk in the song sparrow (*Melospiza melodia*)

ABSTRACT

The adrenocortical stress response may mediate differences in parental effort, with increases in glucocorticoids via the acute stress response facilitating survival and suppressing reproductive activity. However, few studies have investigated whether variation in the stress response is associated with differences in parental risk-taking, or the potential for predation pressure to induce relationships between the stress response and parental behavior. We examined whether parental effort and risk-taking are associated with variation in the stress response and body condition in the song sparrow (*Melospiza melodia*). We predicted that the stress response (elevation of plasma glucocorticoids following exposure to a stressor) negatively, whereas body condition positively, correlates with parental effort and risk-taking. Consistent with predictions, females with smaller stress responses displayed higher nestling stage nest attentiveness. However, female incubation attentiveness, female nestling provisioning rates and male baseline parental effort did not vary as a function of the stress response. Moreover, male, but not female, risk-taking was associated with the stress response, but in a fashion opposite to predictions. Specifically, males with smaller stress responses tended to decrease both offspring provisioning rates and nest attentiveness more in response to adult predator presence than males with larger stress responses, suggesting that these males prioritize self-maintenance over parental care. Baseline corticosterone (the major avian glucocorticoid) and body condition did not correlate with parental effort or risk-taking. Results suggest that the stress response may mediate some dimensions of parental behavior, but that additional mechanisms are involved and that associations vary between the sexes and depend on behaviors measured. *Keywords*: Parental effort, behavioral plasticity, parental risk-taking, adrenocortical stress response, *Melospiza melodia*

INTRODUCTION

Adaptive balance of the survival-reproduction tradeoff (Williams 1966; Levins 1968; Via and Lande 1985; Stearns 1989; Roff 1992) is mediated by proximate control mechanisms that translate environmental stimuli into phenotypic effects (Ricklefs and Wikelski 2002; Zera et al. 2007). In vertebrates, the adrenocortical stress response influences responses to perturbations, including the decision regarding whether to terminate or to maintain reproductive activity (Sapolsky et al. 2000; Romero 2002; Wingfield and Sapolsky 2003; Romero 2004; Wingfield 2008; Bókony et al. 2009; Breuner 2011). During a stress response, steroid hormones known as glucocorticoids (GCs) (corticosterone (CORT) in birds) are released from the adrenal cortex, and plasma concentrations rapidly rise above baseline levels (Sapolsky et al. 2000). The acute stress response facilitates survival and suppresses reproduction by promoting physiological and behavioral changes including stimulation of glycogenolysis and gluconeogenesis, promotion of flight or fight responses, and down-regulation of the hypothalamus-pituitary-gonadal axis and reproductive function (Wingfield et al. 1992; Sapolsky et al. 2000; Romero and Wikelski 2001; Landy et al. 2006; Wingfield 2008). Baseline GCs help the organism cope with predictable energetic demands associated with daily activity cycles and seasonal life-history stages (Landys et al. 2006; Bonier et al. 2009), but concentrations beyond a threshold level may initiate an emergency life-history stage, in which reproductive activity is terminated (Wingfield et al. 1998; Wingfield and Sapolsky 2003; Landys et al. 2006; Romero et al. 2006).

On the interspecific level, adaptation of the stress response allows for appropriate responses to perturbations within the context of a specific life history. Appropriate response may entail down-regulation of the stress response and prioritization of reproduction, for species with low survival probability, low potential for future reproduction, and high brood-value (Bókony et al. 2009). Conversely, species with high survival rates and low brood-value may benefit by maintaining a robust stress response and prioritizing self-maintenance over reproduction (Wingfield 1994; Wingfield et al. 1998; Wingfield and Sapolsky 2003; Wingfield 2005a, b; Breuner et al. 2008). Indeed, in a recent meta-analysis of 64 bird species, Bókony et al. (2009) found that attenuation of the adrenocortical stress response during the breeding season is associated with high brood-value.

On the intraspecific level, differences in adrenocortical stress physiology may also reflect differential investment into reproduction or parental effort. Indeed, numerous studies demonstrate that experimental elevation of GCs can lead to termination of reproductive activities, reduced parental effort, or compromised reproductive success (Wingfield and Silverin 1986; Almasi et al. 2008; Almasi et al. 2013; Thierry et al. 2013). Moreover, a number of factors associated with increased brood-value and reduced opportunities for future reproduction, including advanced age in long-lived species (Heidinger et al. 2006; Heidinger et al. 2010; Wilcoxen et al. 2011), short breeding seasons (Wingfield et al. 1995; Silverin et al. 1997; Breuner and Hahn 2003; Wilson and Holberton 2004), sex (either the female or male is often more parental, and females usually have higher certainty of parentage) (Bókony et al. 2009 for review), experimental enlargement of clutch size (Lendvai et al. 2007), and brood order (broods later in the breeding season increase in value due to decreased opportunity for future reproduction) (Schmid et al. 2013), have all been associated with attenuation of the stress response. However, relatively few studies have examined the relationship between naturally occurring variation in GCs and direct metrics of parental effort (particularly continuous metrics such as offspring provisioning rates or nest defense), perhaps due to logistical challenges of measuring parental behavior in the field (but see Breuner 2011 for review). Further, even fewer studies have examined whether naturally occurring variation in the stress response is associated with differences in parental risk-taking behaviors that entail differential balance of the survival-reproduction tradeoff, or whether predation risk can induce relationships between variation in the stress response and parental effort by elevating costs of parental care and magnifying the tradeoff between survival and reproduction (but see Lendvai and Chastel 2010).

Using the song sparrow (*Melospiza melodia*) as a study species, we explored whether inter-individual variation in the adrenocortical stress response is associated with differences in parental effort and parental risk-taking behavior. The specific objectives of our studies were: (1) to determine whether baseline levels of parental effort (in the absence of elevated predation risk) are associated with variation in the magnitude of the stress response, baseline CORT concentrations, or body condition, (2) to examine whether parental risk-taking behavior is associated to variation in the magnitude of the stress response, baseline CORT concentrations, or body condition, (3) to explore whether elevated costs of parental care induced by predation pressure may induce relationships between stress physiology, body condition, and parental behavior, and finally (4) to determine whether relationships between stress physiology, body condition and parental effort are equivalent, or different, between males and females.

We made specific predictions regarding how levels of parental effort and responses to predation risk would differ as a function of variation in CORT levels and body condition. Firstly, we predicted that individuals with small stress responses (measured by change in CORT (delta CORT, elevated-baseline CORT concentrations) and elevated or acute CORT (maximal level post-stressor)) would display higher parental effort under baseline conditions, and would prioritize offspring wellbeing when responding to predation risk. Conversely, we predicted that individuals with large stress responses would display low parental effort under baseline conditions and would respond to predators in a fashion prioritizing self-maintenance. In addition, we predicted that individuals with lower baseline CORT concentrations would display lower parental effort and willingness to take risks for offspring, since elevated baseline CORT has been related to poor body condition or quality, low reproductive effort and reproductive success, and prioritization of self-maintenance (Landys et al. 2006; Bonier et al. 2009; Angelier et al. 2009, 2010). However, elevation of baseline CORT may also facilitate energetic investment into breeding (Landys et al. 2006; Doody et al. 2008; Bókony et al. 2009; Bonier et al. 2009), in which case baseline CORT may positively reflect parental effort and willingness to take risks for offspring. Finally, we predicted that body condition is positively associated with parental effort and risk-taking for offspring, since high-quality individuals in good condition may be able to invest more into reproduction without jeopardizing survival probability (Drent and Daan 1980). Conversely, given low residual reproductive value (Williams 1966; Roff 1992), individuals in poor condition may terminally invest in reproduction and condition may negatively correlate with parental effort and risk-taking.

We tested these predictions by measuring individual variation in the stress response and body condition and recording behavioral responses to manipulations of perceived nest predation risk during incubation and adult predation risk during the nestling stage. Since only females incubate, focal individuals during incubation were female. However, we considered the behavior of both sexes during the nestling stage. Importantly, increasing incubation attentiveness in response to nest predation risk entails a self-maintenance cost associated with elongated on-bouts, while maintaining reproductive effort in the face of adult predation risk entails survival costs. Thus, behavioral responses to predation risk may reflect differential balance of the survival-reproduction tradeoff. We outline predictions specific to our experimental design in Table 3.1.

METHODS

Study species and site

We studied song sparrows breeding along Convict and McGee creeks, on the eastern slope of the Sierra Nevada (Mono County, CA). Convict Creek flows through the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL; 37º36'51"N/118º49'47"W). McGee Creek is located approximately 10 miles south of SNARL in the Inyo National Forest (37°33'20"N/ 118°47'35"W). We conducted research over the course of three breeding seasons, from early May to mid-August, 2010-2012.

M. melodia is socially monogamous and exhibits biparental care. Females are sole incubators of a 2- to 6-egg clutch, but both males and females provision nestlings (Arcese et al. 2002). Song sparrows in our populations can fledge multiple clutches per season and repeatedly re-nest after clutch loss. Thus, our sample includes repeated measures on nests of the same nesting pair. Predation rates on nests are high. Although we observed few predation events, ermine (*Mustela erminea*) and garter snakes (*Thamnophis* sp.) removed nestlings, and parasitism by brown-headed cowbirds (*Molothrus ater*) was frequent. Corvids, including Western scrub-jays (*Aphelocoma californica*), Steller's jays (*Cyanocitta stelleri*), and black-billed magpies (*Pica hudsonia*), and small mammals, including least chipmunk (*Neotamias minimus*) and deer mice (*Peromyscus maniculatus*) contribute to predation on open-cup passerine nests (including *M. melodia*) in eastern Sierra riparian corridors (Latif et al. 2012).

As in other passerines, *M. melodia* displays seasonal modulation of the stress response (Wingfield 1984), which may aid in coping with diverse life-history demands, such as breeding, migration, and molt (Romero 2002). Both baseline and acute CORT concentrations are high during the breeding season relative to during other life-history stages (Wingfield 1984; Newman et al. 2008). However, as in other species, sustained or frequent elevation of CORT concentrations above seasonal baseline may interfere with reproductive activity. Indeed, male song sparrows in which baseline CORT concentrations were experimentally elevated via silastic implants were less responsive to simulated territorial intrusions, suggesting suppression of reproductive activity (Wingfield and Silverin 1986).

Measuring the adrenocortical stress response and body condition

We used conspecific playback to capture territorial males in mist nets and measured the stress response using standard capture protocol (Wingfield et al. 1992; Wingfield 1994). We caught females either incidentally with males, or via target netting at incubation or nestling stage nests. We controlled for date of capture in statistical analyses. We took an initial blood sample within 3 minutes of capture (representative of baseline CORT) and another sample after 30 minutes (representative of elevated or acute CORT). Birds were confined in cloth holding bags in the interlude between sampling points. We used 26-gauge needles and heparinized microcapillary tubes to withdraw small blood samples (~80 μl) from the brachial vein. We stored blood samples on ice in the field, separated plasma from cell fraction via centrifugation for 12 minutes at 11,000 rpm, and stored plasma at -30°C.

In addition, at the time of capture, we measured body mass (± 0.1 g) using a digital scale, wingchord (± 1 mm) using a wing scale, and tarsus (± 0.01 mm) using digital calipers. We subsequently derived a metric of body condition using residuals of a regression of body mass on wingchord (Albretch et al. 2005).

Predator presentation experiments

Incubation stage: Female responses to offspring-directed predation risk

During incubation we performed a nest predator presentation to assess female responses to perceived nest predation risk (see Table 3.1 for predictions). Nest predator presentations consisted of a taxidermic mount and recording of a Western scrub-jay. A house finch (*Haemorhous mexicanus*) decoy and recording served as a negative control, and we also recorded baseline behavior in the absence of any decoy. We performed scrub-jay, finch, and baseline recordings in randomly determined order for sequential blocks of 2 hours. We used Canon 800 series camcorders to video-record nests. We placed decoys 6 to 10 meters from nests, 2 meters off the ground (Ghalambor and Martin 2000, 2001; Peluc et al. 2008), and projected recordings of vocalizations using an MP3 player placed on the ground below decoys. We placed camcorders 3 to 6 meters from nests and concealed the devices to prevent disturbance originating from recordings alone. We conducted presentations between 0600 and 1400 PDT, and between day 2 and 8 of the incubation period, unless the nest was older when located. We controlled for the effects of stage (day in the incubation period) and time of day in statistical analyses.

Nestling stage: Male and female responses to adult-directed predation risk

During the nestling stage we performed adult predator presentations to assess the willingness of males and females to risk their own survival to provision nestlings (see Table 3.1 for predictions). Adult predator presentations consisted of a taxidermic mount and recording of a sharp-shinned hawk (*Accipiter striatus*). As for nest predator presentations, a house finch decoy and recording served as a negative control, and we also recorded baseline behavior. We conducted adult predator trials as described for nest predator presentations, on day 2 to 8 of the nestling period, unless nests were more advanced when located. We again controlled for stage (day in the nestling period) and time effects in statistical analyses.

Table 3.1. Predicted relationships between parental effort (incubation attentiveness, offspring provisioning rates, nestling stage nest attentiveness), responses to nest predation risk (incubation), and responses to adult-directed predation risk (nestling stage), and the magnitude of the stress response, baseline CORT, and body condition.

Independent Variable	**Hypothesis**	**Predictions for correlation with:**		
		Baseline reproductive effort	Increase in incubation attentiveness given nest predation risk	Decrease in offspring provisioning and nest attentiveness given adult-directed predation risk
Stress response, Acute CORT	Down-regulation promotes reproductive investment	–	–	+
Baseline CORT	High levels reflect poor condition and focus on self maintenance	–	–	+
	High levels reflect investment into energetically demanding breeding activities	+	+	–
Body condition	Good condition reflects high energy reserves and investment into reproduction	+	+	–
	Individuals in poor condition terminally invest into reproduction	–	–	+

Corticosterone radioimmunoassays

We conducted CORT radioimmunoassays (RIA) using a MP Biomedical I^{125} kit (07 - 120103), which has been previously validated for use in *M. melodia* (Newman et al. 2008). We followed kit instructions with the exception that baseline plasma samples were diluted 1:100 with steroid diluent (5 μl plasma: 495 μl diluent) and stressed samples 1:200 (5 μl plasma: 995 μldiluent). We assayed all samples in duplicate, and included a control provided by the kit manufacturer in each assay. We randomly assigned birds to assays. However, all samples from a single bird were run in a single assay. Intra-assay coefficients of variation averaged 10.5%, and inter-assay variation 18.1%.

Permits

The University of California, Riverside's Animal Care and Use Committee (protocol A-20100002E) approved all field techniques. Procedures were additionally authorized by a USGS bird banding permit (23035-F), a California State Collecting permit (SC-11059), a Federal Migratory Bird Collecting Permit (MB22670A-0) and a special use permit from the Inyo National Forest (MLD100008P).

Statistical analysis

Controlling for seasonal and temporal differences in CORT levels

We performed all statistical analyses in R 2.15.2 (R Core Team, 2012). Since CORT levels may be influenced by day in season of capture, nesting stage of capture (early/incubation versus nestling stage), and time of capture, we controlled for these variables before entering CORT into models predicting parental behavior. To determine which variables influenced CORT levels, we examined effects of day in season, stage, and time on all CORT variables in each sex separately.

However, since date of capture and stage of capture were highly correlated in our dataset (Pearson correlation: $r = 0.74$, $P < 0.001$), and we were concerned with independent effects of stage and date on CORT, we first took residuals of a regression of capture date on capture stage to derive a factor for date that was independent of stage. Further, we log transformed baseline and acute CORT and square-root transformed delta CORT to achieve normality of model residuals. After determining significant predictors of CORT, we regressed each CORT variable against significant predictor variables to derive residuals controlled for effects that might otherwise confound results. An independent variable was included in the regression from which residuals were derived if it predicted CORT at the $\alpha = 0.05$ level. In addition, we also controlled body condition for stage of capture by taking the residuals of a regression of body condition on capture stage (early versus nestling), since we had previously determined that body condition declines during the nestling stage (M. Grunst, unpublished data).

In addition, in attempt to further control for stage effects on variation in CORT levels, we repeated analyses while restricting the dataset to birds captured during the nesting stage in which the majority of individuals were captured. Specifically, we repeated analyses within males captured only during the early stage (territory establishment/incubation), when most males were captured. We did not have enough power to run separate analyses within nestling stage males. In contrast, we repeated analyses in females captured during the nestling stage, when most females were captured. We did not have a large enough sample size to run separate analyses within females caught early in the season.

Incubation-stage analyses

To assess the effect of independent variables on incubation attentiveness under baseline conditions and given nest predation risk, we used linear mixed effects models (LMM, lme4 package of R) with a Satterthwaite approximation of degrees of freedom (implemented by R package lmerTest). Since we had separate hypotheses regarding how the independent variables: the stress response (delta CORT and acute CORT), baseline CORT, and body condition, would affect female behavior we constructed independent models for these variables. Each model featured incubation attentiveness (percent time female spent incubating eggs) as a dependent variable. We transformed incubation attentiveness by squaring values. We included treatment (baseline, jay, finch) and the independent variable of interest (delta CORT, acute CORT, baseline CORT, or body condition) in the model as fixed effects. Additionally, we included day in the incubation stage of recording, time of recording, day in season of recording, and clutch size in the model as fixed-effect covariates, and nest number and female and male identity as random effects. Finally, we allowed for an interaction between the independent variable and treatment.

Nestling stage analyses

To assess the effect of independent variables on parental effort during the nestling stage under baseline conditions and given adult predation risk, we also used linear mixed effects models. As for incubation, we had separate hypotheses regarding how the independent variables: the stress response (delta CORT and acute CORT), baseline CORT, and body condition would affect female and male behavior. Thus, we constructed independent models for these variables for both males and females. We had two metrics of parental effort in the nestling stage: offspring provisioning rate and nest attentiveness. Thus, we constructed one set of models with offspring provisioning rate per hour as the dependent variable, and another set of models with nest attentiveness as the dependent variable. We normalized model residual by adding one to offspring provisioning rates and taking the square-root and by squaring nestling stage nest attentiveness. We entered treatment (baseline, hawk, finch) and the independent variable of interest (delta CORT, acute CORT, baseline CORT, or body condition) as fixed effects. Additionally, we included day in the nestling stage, time of recording, day in season of recording, and brood size in each model as fixed-effect covariates, and nest number and male and female identity as random effects. Finally, we allowed for an interaction between the independent variable and treatment. We reduced all models via a step-wise, backwards-elimination process until all explanatory variables remaining were significant (α=0.05).

RESULTS

Plasma CORT levels

Across the sexes, mean ± SE baseline CORT concentrations were 51.17 ± 3.74 ng/ml and acute CORT concentrations were 168.15 ± 8.91 ng/ml. CORT levels after capture stress were significantly higher than baseline levels (Wilcoxon rank sum test, W = 1477, $P < 0.001$). Delta CORT averaged 116.77 ± 7.95 ng/ml.

Males had significantly higher baseline CORT concentrations than females (LMM: $F_{1,130}$ = 35.73, β = 0.78 ± 0.13, $P < 0.001$), with levels averaging 61.87 ± 4.90 ng/ml in males and 28.25 ± 3.10 ng/ml in females. We could detect no effect of capture date, time of capture or stage of capture (early/incubation versus nestling) on baseline CORT concentrations in either males or females (LMM: $P > 0.05$; Appendix 3, Table 3.A1). Since there were no significant predictors of baseline CORT concentrations, baseline CORT alone was used in models of behavior (without taking residuals).

Males also had higher acute CORT levels than females (LMM: $F_{1,98}$ = 36.48, β = 3.56 ± 0.59, $P < 0.001$). Acute CORT averaged 201.89 ± 10.64 ng/ml in males and 93.59 ± 7.46 ng/ml in females. Date and time of capture both negatively correlated with acute CORT across the sexes (LMM: $F_{1,103}$ = 9.18, β = -0.79 ± 0.26, P = 0.003; $F_{1,109}$ = 6.71, β = -0.61 ± 0.24, P =0.01). In males, acute CORT was predicted by capture stage alone (LMM: $F_{1, 57} = 7.92$, $\beta = -0.35 \pm 0.12$, $P = 0.01$), so residuals were taken for use in behavioral models. In females, acute CORT was negatively predicted by residual date (LMM: $F_{1, 10} = 10.39$, $\beta = -0.01 \pm 0.003$, $P = 0.01$) and time (LMM: $F_{1, 29} = 7.42$, $\beta = -0.08 \pm 0.03$, $P = 0.01$), but not by capture stage (LMM: $P > 0.10$; Appendix 3, Table 3.A1), so residuals controlled for the effect of capture date and time.

Finally, delta CORT was similarly higher in males than in females (LMM: $F_{1, 103} = 9.38$, $\beta = 2.31 \pm 0.75$, $P = 0.003$), with values averaging 138 ± 10.02 ng/ml in males and 65.09 ± 6.92 ng/ml in females. Capture stage negatively predicted delta CORT across both sexes (LMM: $F_{1, 108} =5.55$, $\beta = -1.70 \pm 0.72$, $P = 0.02$). In males alone, delta CORT marginally significantly related to nestling stage (LMM: $F_{1, 74} = 3.92$, $\beta = -2.13 \pm 1.08$, $P = 0.051$), but not residual date, or time (Appendix 3, Table 3.A1). Thus, we used residuals of a regression of delta CORT on capture stage in models of male behavior conducted within the entire dataset. In females, delta CORT was negatively predicted by residual time (LMM: $F_{1, 32} = 7.28$, $\beta = -0.46 \pm 0.17$, $P = 0.01$) and marginally significantly related to residual capture date (LMM: $F_{1, 22} = 3.93$, $\beta = -0.04 \pm 0.02$, $P = 0.06$), so we used residuals to control for these effects in behavioral models.

Female incubation behavior

The finch treatment was not significantly different from baseline in this, or any subsequent analysis (LMM: $P > 0.10$; Appendix 3, Table 3.A2), so finch trials were recoded as baseline for use in final models. Females reduced incubation attentiveness in response to the corvid nest predator (LMM: $F_{1, 121} = 7.30$, $\beta = -0.06 \pm 0.02$; $P = 0.01$), and incubation attentiveness decreased later in the day (LMM: $F_{1, 165} = 7.01$, $\beta = -0.01 \pm 0.005$; $P = 0.01$), and later in the incubation stage ($F_{1, 86} = 5.22$, $\beta = -0.01 \pm 0.003$, $P = 0.02$). However, we could find no evidence that baseline, delta, or acute CORT in females affected incubation attentiveness or responses to nest predation risk (LMM: $P > 0.05$ in all cases; Appendix 3, Table 3.A3). We also could find no evidence that female condition modified overall incubation attentiveness or responses to nest predation risk (LMM: $P > 0.10$, Appendix 3, Table 3.A3). When restricting the analysis to females captured during the nestling stage, the pattern emerging was qualitatively the same.

Female parental behavior during the nestling stage

Females reduced offspring provisioning rates in the presence of the adult predator decoy (LMM: $F_{1,\,88} = 10.80$, $\beta = -0.26 \pm 0.08$, $P < 0.001$), and nestling age (LMM: $F_{1,\,131} = 61.69$, $\beta = 0.19 \pm 0.02$, $P < 0.001$) and brood size positively predicted feeding rate (LMM: $F_{1,\,46} = 11.33$, $\beta = 0.20 \pm 0.06$, $P = 0.002$). However, we could find no evidence that plasma CORT concentrations or body condition of females modified offspring provisioning rates either independent of, or in response to, predation risk (LMM: $P > 0.10$; Appendix 3, Table 3.A4). Similarly, when restricting the analysis to females captured during the nestling stage, CORT levels and body condition failed to predict feeding rates (LMM: $P > 0.10$ in all cases).

Females reduced nest attentiveness during the nestling stage in the presence of the predator (Tables 3.2), and nestling age had a strong negative effect on female nest attentiveness (Tables 3.2). Again, we could find no evidence that female CORT levels or body condition modified reduction in nest attentiveness in the presence of the predator (LMM: interaction terms, $P > 0.10$; Appendix 3, Table 3.A5). However, independent of predator presence, females with higher delta (Table 3.2a; Figure 3.1a) and acute CORT (Table 3.2b; Figure 3.1b) tended to be less attentive at nestling stage nests. Baseline CORT concentrations and body condition were not associated with variation in maternal nestling stage attentiveness (LMM: $P > 0.10$; Appendix 3, Table 3.A5). All females for which we obtained measurements of delta CORT and acute CORT as well as

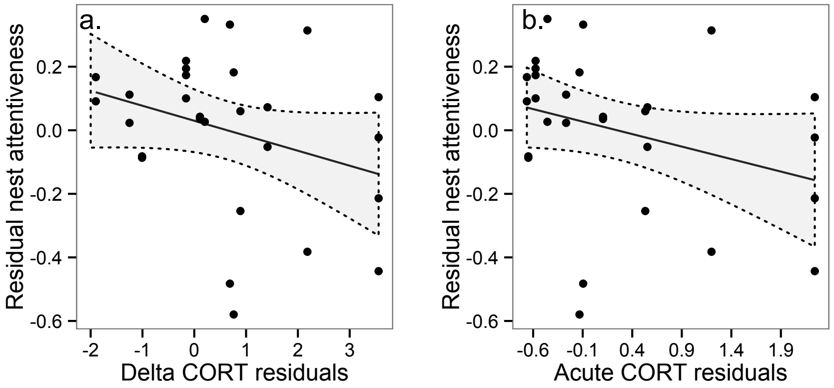

Figure 3.1. Linear regressions of maternal nestling stage nest attentiveness as a function of delta (a) and acute (b) CORT. Delta and acute CORT values are residuals of regressions controlling for capture date. Female attentiveness values are residuals of a regression controlling for nestling age. Shaded regions show 95% confidence intervals.

Table 3.2. Linear mixed effects models predicting female nest attentiveness during the nestling stage from delta and acute CORT

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Delta CORT[a]				
Treatment	-0.24 ± 0.08[b]	9.63	23.99	0.005
Delta CORT	-0.05 ± 0.02	3.59	23.99	0.07
Nestling age	-0.07 ± 0.02	10.33	23.99	0.01
Acute CORT[c]				
Treatment	-0.24 ± 0.08[a]	9.70	23.99	0.002
Acute CORT	-0.08 ± 0.04	3.81	23.99	0.06
Nestling age	-0.10 ± 0.03	10.73	23.99	0.003

[a] N = 28 observations, 14 nests, 12 females, 12 males.
[b] Predator (hawk) treatment relative to baseline.
[c] N = 28 observations, 14 nests, 12 females, 12 males.

nestling stage nest attentiveness were captured during the nestling stage. However, baseline CORT (LMM: $F_{1,8}$ = 0.30, β = 0.0003 ± 0.007, P = 0.60) and body condition still had no effect on nestling stage nest attentiveness when restricting analysis to the nestling stage.

Male parental behavior during the nestling stage

Males reduced offspring provisioning rates in the presence of adult-directed predation risk, and brood size and nestling age positively predicted feeding rate (Table 3.3). Further, males with higher acute CORT reduced provisioning rates less (LMM: treatment × acute CORT interaction term; Table 3.3; Figure 3.2a), and thus tended to display higher provisioning rates in the presence of the predator when controlling for the effects of brood size and nestling age, although the effect of acute CORT on male provisioning rates within the predator treatment alone was not statistically significant (LMM: $F_{1,33} = 2.99$, $\beta = 0.51 \pm 0.30$, $P = 0.09$). The same pattern also tended to emerge in terms of male delta CORT levels, with males with higher delta CORT tending to reduce offspring provisioning rates less in the presence of the predator, although this effect was only marginally significant (LMM: $P = 0.10$; treatment × acute CORT interaction term; Table 3.3; Figure 3.2b), when controlling for the effect of brood size and nestling age. Within the predator treatment alone, delta CORT did not predict offspring provisioning rate, although the trend was toward a positive relationship (LMM: $F_{1,35} = 1.54$, $\beta = 0.04 \pm 0.03$, $P = 0.22$). Males differing in baseline CORT and body condition did not display differences in offspring provisioning rates (LMM: $P > 0.10$; Appendix 3, Table 3.A6). Within early-stage males alone, the models predicting male offspring provisioning rate from acute CORT (LMM: treatment × acute CORT interaction term; $F_{1,64} = 4.00$, $\beta = 0.51 \pm 0.26$, $P = 0.049$) and delta CORT (LMM: treatment × delta CORT interaction term; $F_{1,64} = 2.76$, $\beta = 0.12 \pm 0.07$, $P = 0.10$) were qualitatively the same.

Similar to females, males were less attentive at nests when nestlings were older (LMM: $F_{1,16} = 11.12$, $\beta = -0.04 \pm 0.01$, $P = 0.004$), and attentiveness also increased with date (LMM: $F_{1,16} = 4.77$, $\beta = 0.003 \pm 0.001$, $P = 0.04$). Moreover, males with higher delta CORT were more attentive at nests, independent of the presence of the predator (LMM: $F_{1,16} = 5.59$, $\beta = 0.02 \pm 0.01$, $P = 0.03$). No other variable, including the presence of the predator affected male nest attentiveness (LMM: $P > 0.10$; Appendix 3, Table 3.A7) across males captured in both nesting

Table 3.3. Linear mixed effects models predicting paternal provisioning rates from acute and delta CORT and nest attentiveness from delta CORT among males captured early in the season

	Estimate (β)	F	Denom (df)	P (> F)
Offspring provisioning rate/ acute CORT[a]				
Treatment	-0.32 ± 0.07	21.20	68.92	<0.001
Delta CORT	-0.06 ± 0.28	0.71	33.60	0.41
Brood size	0.28 ± 0.06	20.84	32.66	<0.001
Nestling age	0.10 ± 0.02	25.94	103.54	<0.001
Treatment × delta CORT	0.55 ± 0.23	5.74	68.95	0.02
Offspring provisioning rate/ delta CORT[c]				
Treatment	-0.30 ± 0.07	18.23	68.84	<0.001
Acute CORT	-0.002 ± 0.03	0.47	34.61	0.49
Brood size	0.28 ± 0.06	19.72	30.51	<0.001
Nestling age	0.10 ± 0.02	25.15	103.97	<0.001
Treatment × delta CORT	0.04 ± 0.02	2.64	69.14	0.10
Nest attentiveness[d]				
Treatment	0.01 ± 0.02[b]	0.34	14.23	0.57
Delta CORT	0.01 ± 0.02	3.08	12.39	0.61
Day in stage	-0.03 ± 0.01	8.70	11.75	0.01
Treatment × delta CORT	0.05 ± 0.02	4.41	14.37	0.059

[a] N = 107 observations, 39 nests, 29 males, 30 females.
[b] Predator (hawk) treatment relative to baseline.
[c] N = 31 observations, 15 nests, 14 males, 14 females.

stages. Within early-stage males, instead of delta CORT independently predicting higher nest attentiveness, males with higher delta CORT tended to reduce nest attentiveness less, and thus to display higher nest attentiveness, in the presence of the predator (LMM: nearly significant treatment × delta CORT interaction, Table 3.3b). Indeed, nearly significant positive interactions were also present in initial models predicting paternal nestling stage nest attentiveness from delta and acute CORT in the entire dataset (see Appendix 3, Table 3.A7), but effect became weaker in reduced models. Other models predicting paternal nestling stage nest attentiveness within early-stage males were qualitatively equivalent to models run in the entire dataset, revealing no significant patterns.

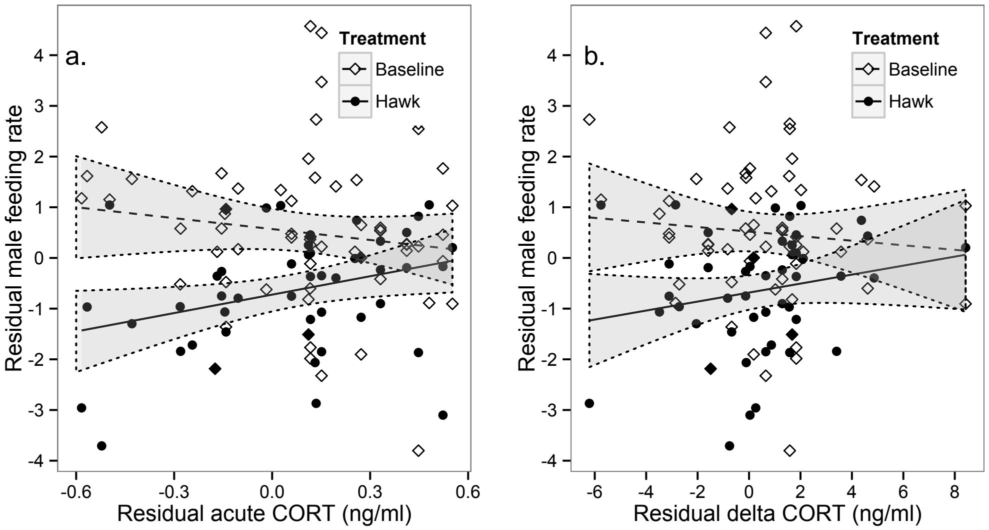

Figure 3.2. Relationships between paternal provisioning rate and acute (a) and delta (b) CORT concentrations under baseline conditions and in the presence of the predator. Shaded regions show 95% confidence intervals. Values for paternal provisioning rate are residuals of a regression that controls for nestling age and brood size. Values for male acute and delta CORT are residuals of a regression of CORT levels on capture stage. Scales differ because acute CORT was log transformed when deriving residuals, whereas delta CORT was square-root transformed.

DISCUSSION

The stress response and parental effort

Our results are largely inconsistent with past empirical work suggesting that attenuation of the adrenocortical stress response may help mediate investment into parental effort (Romero 2002; Wingfield and Sapolsky 2003; Landys et al. 2006). The only support for the idea that lower stress responses may facilitate increased parental effort derived from the relationship between CORT concentrations and maternal nestling stage nest attentiveness. Specifically, in female song sparrows both the magnitude of the stress response (delta CORT) and absolute levels of CORT after stress (acute CORT) tended to be negatively associated with nestling stage nest attentiveness (Wingfield 1994; Wingfield et al. 1998; Wingfield and Sapolsky 2003; Bókony et al. 2009). However, the negative relationship between acute and delta CORT in females and nestling stage nest attentiveness was not statistically significant ($P = 0.06$ and $P = 0.07$), and our sample sizes were low. Thus, results must be interpreted with caution.

Moreover, we could detect no effect of delta or acute CORT on female offspring provisioning rates or incubation attentiveness. Thus, even if suppression of the stress response contributes to up-regulation of some parental behaviors, additional mechanisms must also contribute to variation in parental effort in females. Indeed, although some studies have reported that smaller magnitude stress responses are associated with increased brood-value and increased parental effort (Pereyra and Wingfield 2003; Lendvai et al. 2007; Lendvai and Chastel 2008, 2010), others have failed to find a relationship, or have even reported positive relationships (Chastel et al. 2005; DuRant et al. 2013). For instance, in house sparrows (*Passer domesticus*) birds with experimentally enlarged broods displayed reduced acute CORT levels relative to controls (Lendvai et al. 2007), supporting the idea that the stress response is down-regulated to enhance parental investment. However, in black-legged kittiwakes (*Rissa tridactyla)* breeding status (chick-rearing versus failed) was not associated with differences in the magnitude of the stress response, but rather was associated with differences in how prolactin levels changed in response to stress (Chastel et al. 2005), with chick-rearing individuals displaying smaller decreases in prolactin levels. Although not proving a causative link, this result suggests that prolactin, rather than CORT, may mediate differences in parental effort in this species.

Further, rather than detecting negative relationships between delta or acute CORT on parental effort in males, we detected only positive relationships. Specifically, males with higher, not lower delta CORT displayed higher nestling stage nest attentiveness, and males with higher delta and acute CORT also tended to reduce offspring provisioning rates less in response to adult-directed predation risk. Thus, results suggest that the stress response may differentially mediate parental behavior in the two sexes. This contingency is in opposition to a number of interspecific studies that have found that reduced stress responses correlate to higher parental contribution in both males and females (Meddle et al. 2003; Bókony et al. 2009), and intraspecific studies that report that elevating CORT decreases parental effort in both sexes (Wingfield et al. 1998; Bókony et al. 2009). Nevertheless, a recent review reports that acute CORT levels are lower in females of avian species with female-biased parental care, but also lower in females of species with male-biased care (Bókony et al. 2009). Thus, in least in some species, males may be selected to retain larger stress responses than females, but may be able to resist negative impacts of elevated CORT on breeding and paternal care (Meddle et al. 2003; Wingfield and Sapolsky 2003), perhaps via modulation of down-stream mechanisms such as receptor or binding-globulin numbers (Breuner and Orchinik 2002; Meddle et al. 2003).

However, differential effects of CORT on parental care in males and females in this study may also have arisen because, on average, we captured males and females at different times during the breeding season. Specifically, we captured most females during the nestling stage, but most males earlier in the season. Thus, differences in the stress response in females may be more reflective of nestling stage differences in parental effort. Males with higher early-season stress responses may have down-regulated the stress responses at the onset of paternal activity, equalizing or changing patterns of hormonal variation across males and facilitating paternal investment (Holberton and Wingfield 2003; Wingfield and Sapolsky 2003).

The stress response and behavioral responses to predators

Our results provide some support for the idea that the stress response may mediate responses to predation risk, and that elevated costs of parental care associated with predator presence may induce differences in parental behavior (Lendvai and Chastel 2010). Specifically, although we could find no evidence that CORT levels reflected risk-taking in females, males with higher, not lower acute CORT (and to some extend delta CORT) reduced offspring provisioning rates less in response to the predator compared to males with lower CORT levels. In addition, within males captured early in the season, birds with higher delta CORT tended to reduce attentive at nestling stage nests less in the presence of the predator compared to males with lower delta CORT (although this effect did not emerge within the entire dataset). Greater reductions in offspring provisioning rates in response to the predator in males with low acute CORT concentrations produced a nearly significant positive relationship between acute CORT and provisioning rates under predation risk that was not present under baseline conditions. These results are similar to Lendvai and Chastel's (2010) finding that acute CORT in house sparrows is correlated with nestling provisioning rates after, but not before, a stressor. However, in our study, males with lower acute CORT tended to reduce offspring provisioning rates more in response to the predator than males with higher acute CORT. This result is inconsistent with the prediction that lower acute or delta CORT mediates higher investment into parental effort, and should thus correlate with lesser reductions in parental care in the face of adult predation risk (Wingfield and Sapolsky 2003; Lendvai and Chastel 2010). Indeed, in contrast to our results, Lendvai and Chastel (2010) found that house sparrows with lower acute CORT fed offspring less after exposure to a stressor, suggesting that down-regulation of the stress response supports sustained reproductive effort in the context of perturbation.

Our results may be explained by the fact that we captured most males early in the season, such that CORT levels in males may not mechanistically relate to levels of paternal care. Rather, males with higher early-season stress responses may down-regulate the stress response at the onset of paternal activity to facilitate paternal care, as suggested above. However, this explanation falls short of explaining why reductions in feeding rate in the presence of the predator would negatively correlate with acute CORT (Holberton and Wingfield 2003; Wingfield and

Sapolsky 2003). One possibility is that males with high early-season stress responses are high quality birds that invest highly in territorial defense, and also have high paternal capability. In support of this hypothesis, in our population, early season delta and acute CORT positively correlates with song repertoire size, and males with larger repertoires provide more paternal care (M. Grunst, unpublished data). In addition, males with large stress responses may be more prepared to evade predation risk, and may thus be able to sustain paternal behavior in the face of predation risk, while suffering lower costs.

Baseline CORT, body condition and parental behavior

We found no evidence that baseline CORT concentrations are associated with differences in baseline parental effort, females' responses to nest predation risk, or male and female responses to adult-directed predation risk. In contrast to this result, other research has reported correlations between baseline CORT concentrations and either parental effort or reproductive success (which may or may not result from higher parental effort), with both positive (Doody et al. 2008; Bonier et al. 2009; Ouyang et al. 2010; Riechert et al. 2012) and negative relationships observed (Silverin 1986; Kitaysky et al. 2001; Pereyra and Wingfield 2003; Almasi et al. 2008). For instance, in the tree swallow (*Tachycineta bicolor*) females with high breeding-season baseline CORT levels had higher reproductive success (Bonier et al. 2009), perhaps because up-regulation of baseline CORT supports energetic demands of breeding (Landys et al. 2006). Further, in the house sparrow, females with low baseline CORT before, but high baseline CORT during, the breeding season had the highest reproductive success (Ouyang et al. 2010), perhaps because elevated baseline CORT before the breeding season reflects poor condition and quality, while elevated CORT during the breeding season supports energetic demands (Landys et al. 2006; Bonier et al. 2009; Breuner 2011). On the other hand, some studies have also report that baseline

CORT concentrations do not reflect current parental effort or reproductive success (Lendvai et al. 2007; Lendvai and Chastel 2010; Done et al. 2011), perhaps because individuals that invest highly in breeding are well prepared for the energetic demands of parental care (Lendvai et al. 2007). In terms of responses to predators, delta and acute CORT may be more powerful predictors of behavior than baseline CORT since binding of CORT to type II glucocorticoid receptors, which bind CORT only at elevated levels, may mediate patterns of behavioral change in the context of stress (Breuner and Orchinik 2001, 2002).

We could detect no effect of female or male condition on paternal effort. Our failure to find conclusive effects of either baseline CORT or condition on parental effort suggests that energetic stress associated with predictable demands of breeding may not tightly mediate differences in parental effort in our population. Further, body condition may be decoupled from parental effort in our species because maintaining lighter mass may have selective advantages, such as increased maneuverability, especially during the nestling stage when frequent foraging trips must be made. Thus, lighter mass may not necessarily reflect energetic stress (Freed 1981; Cichon 2001; Suarez et al. 2005).

CONCLUSIONS

Our results are largely inconsistent with the hypothesis that suppression of the adrenocortical stress response during the breeding season may mediate increased investment into parental care. Rather, we found only weak support for lower stress responses being associated with increased parental effort in females. Further, we actually observed the opposite pattern in males, with acute and delta CORT concentrations, if anything, positively correlating with paternal effort. Results do yield some support for the idea that a relationship between parental behavior and stress physiology may emerge only in the context of stress, such that fitness ramifications and evolutionary processes linked to variation in CORT levels may be accentuated in the context of stress. However, again, the pattern we observed was sex-specific, emerging in males only. Moreover, the pattern observed was opposite to our predictions with higher, not lower, levels of acute and delta CORT tending to be associated with higher parental effort under predation risk. Thus, our results suggest that the relationship between parental effort and CORT levels may differ between the sexes, and that smaller stress responses may not translate into increased levels of parental effort in our species, especially in males.

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CHAPTER 4: Sex-specific modulation of the adrenocortical stress response, parental effort and parental risk-taking behavior in the song sparrow (*Melospiza melodia*)

ABSTRACT

Even in biparental species, one sex often provides the majority of parental care or has more invested in offspring wellbeing. Thus, theory predicts sex-based differences in the adrenocortical stress response, a physiological mechanism that diverts energy expenditure away from reproductive activity and into self-maintenance. Specifically, the sex with more invested in offspring may down-regulate the stress response more than the other, either across the entire breeding season, or during uniparental periods. Sexes differences in the stress response may equalize during biparental periods, but if differences persist, the more parental sex may display higher parental effort and greater willingness to take risks for offspring even when both males and females are participating in care giving. We evaluated these contingencies by assessing inter-sex differences in plasma corticosterone (the major avian stress hormones), body condition, and parental risk-taking in song sparrows (*Melospiza melodia*). We measured parental risk-taking by experimentally elevating perceived adult-directed predation risk near nests during the biparental nestling period. Consistent with predictions, females, which alone incubate, invest highly in ova and have higher certainty of parentage, displayed lower plasma corticosterone levels. Further, the stress response declined in both sexes during the parentally demanding nestling stage, but remained higher in males. Both sexes similarly decreased feeding rates in response to predation risk and females decreased nest attentiveness in response to predators more than males. However, females displayed higher provisioning rates and nest attentiveness across contexts, suggesting acceptance of greater risk. Body condition declined during the nestling stage in both sexes, with steeper declines in females. However, males were in poorer body condition than females early in the season, and sex did not predict body condition during the nestling stage. Thus, change in body condition may reflect adaptive modulation of body mass rather than self-maintenance costs. *Keywords*: Adrenocortical stress response, parental risk-taking, body condition, inter-sex differences

INTRODUCTION

Iteroparous organisms facing energy limitation encounter a tradeoff between maximizing reproductive success and maximizing survival (Williams 1966; Roff 1992; Stearns 1992). In vertebrates, modulation of the adrenocortical stress response via the hypothalamus-pituitary-adrenal (HPA) axis results in release of glucocorticoids (GCs) from the adrenal cortex and mediates balance of this tradeoff (Wingfield and Sapolsky 2003). Elevation of baseline GC levels may support coping with energetic demands of breeding, but beyond a threshold level may trigger an emergency response and divert energy toward self-maintenance (Landys et al. 2006; Bonier et al. 2009). Further, elevation of GCs in response to acute stressors is generally thought to suppress reproductive activity and promote survival responses (Romero 2002; Wingfield and Sapolsky 2003). Thus, species or individuals with higher brood value, either due to lower expectation of future reproduction or higher clutch or brood size, may down-regulate the stress response more during periods of parental care (Bókony et al. 2009).

Sex often determines parental roles. Thus, since down-regulation of the stress response may promote investment into parental care over self-maintenance activities, the sex responsible for the majority of care giving may suppress the stress response more than the opposite sex (Wingfield 1984; Wingfield et al. 1995; Wingfield and Sapolsky 2003; O'Reilly and Wingfield 2005; Bókony et al. 2009). In birds parental care is often female biased, with females alone incubating eggs, but both sexes contributing to nestling provisioning (Lack 1968). Moreover, due to the prevalence of extra-pair copulations in socially monogamous songbirds, females have higher certainty of parentage, and are thus expected to invest more in care of the social brood (Møller and Thornhill 1998; Westneat and Stewart 2003). Indeed, extensive work has explored sex differences in modulation of the stress response across the breeding season, and in species including white-crowned sparrows (*Zonotrichia leucophyrs*) (Wingfield et al. 1982, 1995; Wingfield 1988), yellow warblers (*Setophaga petochia*) (Wilson and Holberton 2004) and song sparrows (*Melospiza melodia*) (Wingfield 1984), females have lower breeding season stress responses than males.

However, despite well-documented differences in sex differences in the stress response, few studies have jointly examined sex specific modulation of the stress response during the breeding season and differences in parental effort and risk-taking behavior. Doing so represents an essential step in establishing a mechanistic link between modulation of the stress response and sex differences in parental investment, with lower GC levels predicted to promote higher parental effort and greater risk-taking for offspring (Romero 2002; Wingfield and Sapolsky 2003). Moreover, since survival threats activate the stress response and elevate stress hormone levels, parental risk-taking behavior may be especially likely to be linked to variation in stress-induced GC levels (Lendvai and Chastel 2010). Thus, we adopted the novel approach of simultaneously examining sex-specific differences in the stress response and parental effort and risk-taking in breeding song sparrows.

Our study had two specific objectives. Firstly, we strove to document sex-specific patterns of change in plasma corticosterone (CORT, the primary avian GC) concentrations and, as a corollary, to determine whether changes in body condition across the breeding season are associated with sex differences in CORT levels. To clarify, loss of body mass may occur as a consequence of high reproductive investment, and may translate into reduced parental survival probability (Ricklefs 1974; Askenmo 1977; Bryant 1979). Thus, condition declines may be viewed as an indirect metric of costs of reproduction, and may be higher in the more parental sex (Reznick 1985; Roff 1992; Stearns 1992). With respect to this objective, we predicted that female song sparrows display lower plasma CORT concentrations and larger, breeding-season declines in body condition than males, since females incubate, invest highly in eggs, and have higher certainty of parentage. However, during the biparental and parentally demanding nestling period, we predicted that plasma CORT levels would equalize between males and females, and that body condition and CORT levels would decline in both sexes.

Secondly, we wanted to determine whether sex differences in parental effort and risk-taking were in the direction predicted based on CORT levels. As an extension, we also explored whether sex differences in parental care and risk-taking depend on nestling age or brood size, which may modify brood value and optimal levels of parental investment. If male and female CORT levels equalize during the nestling stage, we predicted that parental effort and risk-taking would be similar between the sexes. Alternatively, if lower CORT levels in females persist into the nestling stage and mediate higher reproductive investment, we predicted that females would display higher parental effort and take greater risks for the benefit of offspring (Montgomerie and Weatherhead 1988; Wingfield et al. 1982, 1995). As a final contingency, predator presence might induce sex differences in parental effort, especially if differences in the stress response persist into the nestling stage. Specifically, males and females may display comparable parental effort under baseline conditions when costs of care are low, but males may decrease parental care more steeply when faced with elevated costs of care associated with predation risk (Winkler 1992; Gabor et al. 2000).

METHODS

Study system

Our study population of song sparrows breeds along Convict and McGee Creeks, on the eastern slope of the Sierra Nevada (Mono County, CA). Convict Creek flows through the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL; 37º36'51"N / 118º 49'47"W). McGee Creek is located ~10 miles south of SNARL in the Inyo National Forest (37°33'20"N/ 118°47'35"W). Both study sites are located at mid-elevation between 7,000 and 8,000 feet (2,100 - 2,500 meters). We conducted research across three breeding seasons, from early May through early August, 2010 - 2012.

M. melodia is socially monogamous and exhibits biparental care (Arcese et al. 2002). Females are sole incubators of a 2- to 6-egg clutch, but males may provide limited parental assistance during the incubation stage by engaging in nest-guarding behavior. Both males and females provision nestlings (Arcese et al. 2002). Song sparrows can fledge multiple clutches per season and repeatedly renest after clutch loss.

The University of California, Riverside's Institutional Animal Care and Use Committee (protocol A-20100002E) approved all field techniques and we obtained a USGS bird banding permit (23035-F), a California State Collecting permit (SC-11059), a Federal Migratory Bird Collecting Permit (MB22670A-0) and a special use permit from the Inyo National Forest (MLD100008P) to authorize all methods employed.

Capturing focal individuals and locating nests

We captured focal individuals during two time periods. Firstly, we captured early-season males in mist nests using conspecific playback. We captured early-stage females via weak responses to playback, incidentally, or at incubation-stage nests. Secondly, we captured a smaller number of males, and the majority of females at nests during the nestling stage. Our sample sizes are unequal, with most males captured early and most females captured during the nestling stage, but were sufficient to test for stage and date effects on CORT levels in both sexes. Upon capture, we marked all individuals with a USGS aluminum band and an additional unique combination of three colored leg bands.

We located nests of focal pairs using a combination of systematic searching and behavioral observation (Martin and Geupel 1993). We monitored nests via nest checks every 2 to 3 days until the nest was depredated or fledged. We knew the nesting status of all individuals included in our sample.

Measuring plasma CORT levels and body condition metrics

Upon capture of birds in mist nets, we measured the stress response using standard capture protocol (Wingfield 1994; Wingfield et al. 1992). We took an initial blood sample within 3 minutes of capture (to characterize baseline CORT levels) and another sample after 30 minutes (to characterize CORT levels after acute stress). We confined birds in cloth holding bags in the interlude between sampling points. We used 26-gauge needles and heparinized microcapillary tubes to withdraw small blood samples (~80 μl) from the brachial vein. Blood samples were stored on ice in the field. Plasma was separated from cell fraction via centrifugation for 12 minutes at 11,000 rpm, and plasma stored at -30°C.

In addition, after obtaining the final blood sample, we measured body mass to the nearest 0.1 g using a digital scale, unflattened wingchord to the nearest mm using a wing scale, and tarsus length to the nearest 0.01 mm using digital calipers. We subsequently calculated body condition using residuals of a regression of body mass on wingchord (Albretch et al. 2005).

Baseline reproductive effort and predator presentations

We recorded nestling provisioning rates in both the presence and absence of a taxidermic mount and recording of a sharp-shinned hawk (*Accipiter striatus*). A house finch (*Haemorhous mexicanus*) decoy and recording served as a negative control. We performed hawk, finch, and baseline recordings in randomly determined order for sequential blocks of 2 hours. We video-recorded parental behavior using Canon 800 series camcorders placed 3 to 6 meters from nests and concealed to prevent disturbance originating from the recording alone. We placed decoys 6 to 10 meters from nests, 2 meters off the ground (Ghalambor and Martin 2000, 2001; Peluc et al. 2008), and projected recordings of vocalizations using a MP3 player placed on the ground below decoys. We conducted presentations between 0600 and 1400 PDT on day 6 to 12 of the nestling period. We extracted two metrics of parental effort from video-recordings: offspring provisioning rates (number of trips to the nest delivering food items) and nest attentiveness (percent time spent on the nest).

Corticosterone radioimmunoassays

We conducted corticosterone radioimmunoassays using a MP Biomedical I^{125} radioimmunoassay (RIA) kit (07 - 120103), which has been previously validated for use in *M. melodia* (Newman et al. 2008). We followed kit instructions, with the exception that we diluted baseline plasma samples 1:100 with steroid diluent (5 μl plasma: 495 μl diluent), and stressed samples 1:200 (5 μl plasma: 995 μl diluent). We assayed all samples in duplicate, and included a control provided by the kit manufacturer in each assay. All samples from a single bird were run in a single assay. Intra-assay coefficients of variation averaged 10.5% and inter-assay variation 18.1% (N = 11 assays).

Statistical analyses

We used R 2.15.2 (R Core Team, 2012) to perform statistical analyses. Firstly, to explore intersexual differences in modulation of the stress response across the breeding season we used linear mixed effects models (LMM, lme4 package in R) with baseline CORT, delta CORT (acute CORT minus baseline CORT), acute CORT, and condition as dependent variables in separate models. We log transformed baseline and acute CORT and square-root transformed delta CORT to achieve normality of residuals. We included individual sex, nesting stage of capture, date of capture, and time of capture in each model. However, since date of capture and stage of capture were correlated in our dataset (Spearman correlation: $r_s = 0.73$, $N = 137$, $P < 0.001$) and we were interested in independent effects of capture stage and capture date on CORT levels, we first took the residuals of a regression of capture date on capture stage to derive a factor for date that was independent of stage (here after referred to as residual date). Additionally, to examine sex-specific modulation of the stress response and condition across the breeding season, we included interaction terms between sex and nesting stage and sex and residual date. Finally, we analyzed each capture stage separately (early versus nestling) to further determine whether date of capture had an effect on the stress response and body condition within each nesting stage, and if sex differences persisted within each stage. We included individual as a random effect in all models since we captured some individuals repeatedly, either between or within seasons.

Secondly, we also conducted within-sex analyses to determine whether plasma CORT levels and body condition within males and females vary differently as a function of either the date or nesting stage of capture. These models were identical to the ones described above except for the absence of sex as a main effect and associated interaction terms.

Finally, to explore sex-specific differences in baseline reproductive effort and responses to adult-directed predation risk, we used linear mixed effects models with offspring provisioning rate and nestling stage nest attentiveness as the dependent variable in separate models, and sex, treatment (baseline, finch, hawk), brood size, and day in stage as fixed effects. We provided for interactions between sex and treatment, sex and brood size, and sex and day in stage. We included male and female identity and nest number as random effects. We square-root transformed offspring provisioning rate to normalize residuals. We employed a Satterthwaite approximation (implemented by the lmerTest package of R) to estimate degrees of freedom for linear mixed effects models.

RESULTS

Intersexual differences in plasma CORT levels across the breeding season

Males had significantly higher baseline plasma CORT concentrations than females (LMM: $F_{1, 130} = 35.73$, $\beta = 0.78 \pm 0.13$, $P < 0.001$), with mean ± SE levels in males averaging 61.87 ± 4.90 ng/ml and levels in females averaging 28.25 ± 3.10 ng/ml (Figure 4.1a). However, baseline CORT did not change significantly with nesting stage, residual date, or time of capture, and there was consequently no indication that sex modified changes in baseline CORT concentrations with nesting stage or capture date (LMM: $P > 0.10$, Figure 4.2a; Appendix 4, Table 4.A1). Males also had higher delta CORT than females (LMM: $F_{1, 104} = 9.38$, $\beta = 2.31 \pm 0.75$, $P = 0.003$) with levels in males averaging 138 ± 10.02 ng/ml and levels in females averaging 65.09 ± 6.92 ng/ml (Figure 4.1b). In addition, delta CORT was lower in the nestling stage (LMM: $F_{1, 109} = 6.03$, $\beta = -1.91 \pm 0.78$, $P = 0.02$, Figure 4.2b). Residual date and time of day did not affect delta CORT (LMM: $P > 0.10$; Appendix 4, Table 4.A1). Similarly, males had higher acute CORT than females (LMM: $F_{1, 109} = 31.98$, $\beta = 0.61 \pm 0.11$, $P < 0.001$), with levels in males averaging 201.89 ± 10.64 ng/ml and levels in females averaging 93.59 ± 7.46 ng/ml (Figure 4.1c). Acute CORT was lower during the nestling stage (LMM: $F_{1, 82} = 8.97$, $\beta = -0.28 \pm 0.09$, $P = 0.004$,

Figure 4.2c) and declined with residual date (LMM: $F_{1,112} = 6.07$, $\beta = -0.01 \pm 0.002$, $P = 0.02$) and time of capture (LMM: $F_{1,111} = 11.36$, $\beta = -0.06 \pm 0.02$, $P = 0.001$). There was no indication of interactions between sex and other independent variables in predicting delta or acute CORT levels (LMM: $P > 0.10$; Appendix 4, Table 4.A1).

When early-stage captures were analyzed separately, females still displayed lower CORT levels than males (LMM: baseline CORT, $F_{1,84} = 19.15$, $\beta = 0.94 \pm 0.21$, $P < 0.001$; acute CORT, $F_{1,72} = 17.36$, $\beta = 0.66 \pm 0.16$, $P < 0.001$; delta CORT, $F_{1,72} = 4.80$, $\beta = 2.79 \pm 1.28$, $P = 0.03$). However, date and time of capture were not related to CORT levels within birds captured during the early stage, and there was no indication of date interacting with sex (LMM: $P > 0.10$; Appendix 4, Table 4.A2).

Similarly, differences in CORT levels between the sexes persisted if restricting the analysis to nestling stage captures (LMM: baseline CORT, $F_{1,38} = 6.63$, $\beta = 0.55 \pm 0.21$, $P = 0.01$; acute CORT, $F_{1,39} = 18.57$, $\beta = 0.55 \pm 0.13$, $P < 0.001$; delta CORT, $F_{1,37} = 5.99$, $\beta = 2.12 \pm 0.87$, $P = 0.02$). In addition, within nestling stage captures, both delta and acute CORT declined with time of capture (LMM: delta CORT, $F_{1,37} = 8.26$, $\beta = -0.56 \pm 0.19$, $P = 0.01$; acute CORT, $F_{1,39} = 10.52$, $\beta = -0.09 \pm 0.03$, $P = 0.002$) and capture date (LMM: delta CORT, $F_{1,37} = 6.81$, $\beta = -0.07 \pm 0.03$, $P = 0.01$; acute CORT, $F_{1,39} = 11.66$, $\beta = -0.01 \pm 0.004$, $P = 0.001$). There was no indication of interactions between sex and date when predicting CORT levels within the nestling stage (LMM: $P > 0.10$; Appendix 4, Table 4.3A).

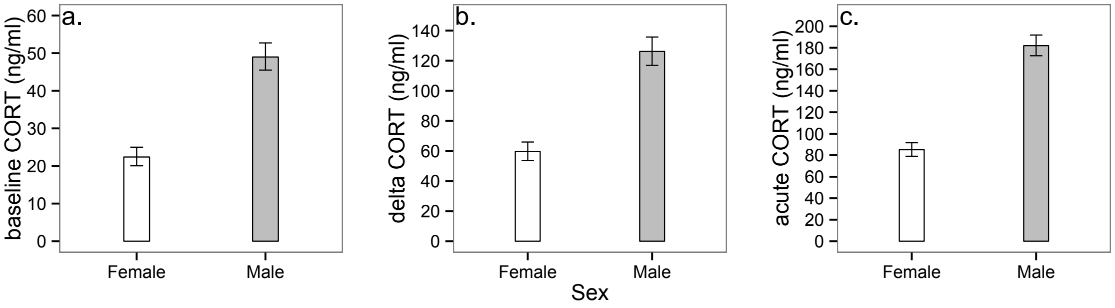

Figure 4.1. Sex differences in CORT levels in song sparrows. Females had lower baseline (a), delta (b) and acute (c) CORT than males. Note the differences in y-axis scales. Bars represent back-transformed means and error bars denote standard error.

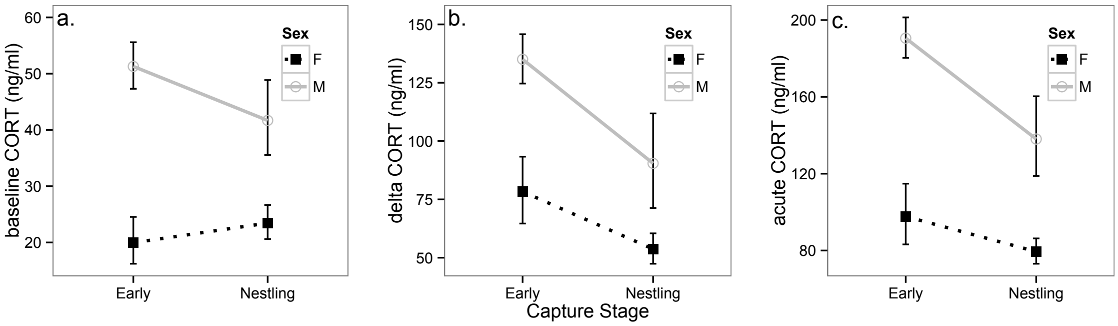

Figure 4.2. Differences in CORT levels with respect to sex and nesting stage of capture. Males had higher baseline CORT than females across nesting stages (a). Males also displayed higher delta CORT, and delta CORT was lower in both sexes in the nestling stage (b). Finally, males had higher acute CORT, and acute CORT also declined in both sexes during the nestling stage (c). Values shown are back-transformed means with bars representing standard error.

Intersexual differences in condition across the breeding season

Across both nesting stages, males were in poorer body condition than females (Table 4.1). In addition, condition was lower in the nestling stage than early in the season in both sexes, and body condition increased with residual date (Table 4.1). The interaction between residual date and sex was not significant (LMM: $F_{1, 72} = 1.65$, $\beta = -0.01 \pm 0.01$, $P = 0.20$). Finally, sex and nesting stage interacted to predict body condition, reflecting a greater decline in female condition between the early and nestling stage (Table 4.1). Time of capture had no effect on condition (LMM: $F_{1,81} = 1.12$, $\beta = -0.04 \pm 0.04$, $P = 0.29$).

Table 4.1. Linear mixed effects model predicting individual body condition from sex, residual date, and capture stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Sex	-1.20 ± 0.35[a]	4.53	116.01	0.04
Capture stage	-2.07 ± 0.37[b]	16.49	81.24	<0.001
Residual date	0.01 ± 0.005	3.65	78.17	0.01
Sex × Capture stage	1.21 ± 0.46	3.41	83.13	0.01

[a] Males relative to females.

[b] Nestling stage relative to early stage.

Within early-stage birds alone, females were in better condition than males (LMM: $F_{1,68} = 8.78$, $\beta = -1.07 \pm 0.36$, $P = 0.004$; Figure 4.3) and birds captured earlier in the season were in better condition (LMM: $F_{1,73} = 11.24$, $\beta = 1.41 \pm 0.45$, $P = 0.002$).

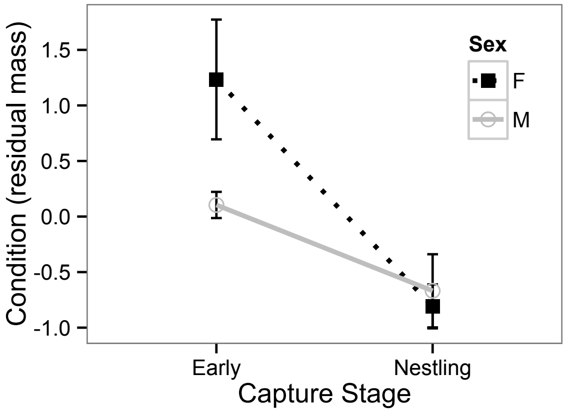

Figure 4.3. Differences in body condition with respect to sex and capture stage. Females were in better body condition than males early in the season. However, although condition declined in both sexes during the nestling stage, the decline was steeper in females such that no inter-sexual difference in condition existed during the nestling stage. Values represent means and bars represent standard error.

Further, sex and capture date interacted negatively to predict condition, reflecting greater declines in female condition with date of capture (LMM: $F_{1,73} = 7.30$, $\beta = -1.26 \pm 0.47$, $P = 0.01$). Time of capture was not related to condition within early-stage birds (LMM: $F_{1,48} = 1.40$, $\beta = -0.06 \pm$

0.05, $P = 0.24$). Body condition did not differ between the sexes (LMM: $F_{1, 35} = 0.002$, $\beta = -0.02 \pm 0.43$, $P = 0.97$; Figure 4.3) or change as a function of capture date or time within nestling stage birds alone (LMM: $P > 0.10$; Appendix 4, Table 4.A4).

Plasma CORT levels and condition within the sexes

In males analyzed separately, baseline CORT concentrations did not change with nesting stage, residual date, or time (LMM: $P < 0.10$; Appendix 4, Table 4.A5). In contrast, delta (LMM: $F_{1, 74} = 3.93$, $\beta = -2.13 \pm 1.08$, $P = 0.05$) and acute CORT (LMM: $F_{1, 57} = 7.92$, $\beta = -0.35 \pm 0.12$, $P = 0.01$) were lower in the nestling stage relative to early in the season, but residual date and time had no predictive power (LMM: $P > 0.10$; Appendix 4, Table 4.5A). Finally, males were in poorer condition during the nestling stage (LMM: $F_{1, 33} = 9.12$, $\beta = -0.81 \pm 0.27$, $P = 0.004$), but male condition was unrelated to capture date or time of capture (LMM: $P > 0.10$; Appendix 4, Table 4.A5).

In females analyzed separately, there were no significant predictors of baseline CORT levels (LMM: $P > 0.10$; Appendix 4, Table 4.A6). Females had lower delta CORT later in the day (LMM: $F_{1, 32} = 7.28$, $\beta = -0.46 \pm 0.17$, $P = 0.01$), and tended to have lower delta CORT later in the season (LMM: $F_{1, 22} = 3.93$, $\beta = -0.04 \pm 0.02$, $P = 0.06$), but nesting stage of capture did not affect female delta CORT levels (LMM: $P > 0.10$; Appendix 4, Table 4.A6). Similarly, females had lower acute CORT later in the season (LMM: $F_{1, 10} = 10.39$, $\beta = -0.01 \pm 0.003$, $P = 0.01$) and later in the day (LMM: $F_{1, 29} = 7.42$, $\beta = -0.08 \pm 0.03$, $P = 0.01$), but acute CORT did not differ with capture stage (LMM: $P > 0.10$; Appendix 4, Table 4.A6). Finally, female condition was lower during the nestling stage (LMM: $F_{1, 35} = 19.35$, $\beta = -2.05 \pm 0.47$, $P < 0.001$). In addition, females captured later in the season tended to be in better body condition, and females captured later in the day tended to be in poorer condition, but these effects were not statistically significant (LMM: $P > 0.05$; Appendix 4, Table 4.A6).

Parental effort and responses to adult predation risk

Females provisioned nestlings at higher rates than males (Table 4.2, Figure 4.4a). Further feeding rate increased with brood size and nestling age, and declined in the presence of the predator (Table 4.2). The finch treatment was not significantly different than baseline (LMM: $P > 0.10$; Appendix 4, Table 4.A7) so we combined the finch and baseline trials for the purpose of the final analysis.

Table 4.2. Linear mixed effects models predicting offspring provisioning rates and nestling stage nest attentiveness from sex, predator presence, brood size and nestling age

	Estimate ($\beta \pm$ SE)	F	Denom (df)	P (> F)
Offspring provisioning[a]				
Sex	-0.15 ± 0.05[b]	7.92	217.43	0.005
Treatment	-0.21 ± 0.05[c]	16.09	219.53	<0.001
Brood size	0.21 ± 0.06	25.87	42.16	<0.001
Day in stage	0.37 ± 0.04	62.09	211.86	<0.001
Brood size × sex	0.11 ± 0.05	4.29	217.43	0.001
Day in stage × sex	-0.18 ± 0.05	11.46	217.43	0.040
Nest attentiveness[d]				
Sex	-0.47 ± 0.04[b]	3.46	73.72	<0.001
Treatment	-0.21 ± 0.04[c]	0.31	74.26	<0.001
Nestling age	-0.05 ± 0.01	0.27	21.69	0.002
Sex × Treatment	0.27 ± 0.06	0.21	73.72	<0.001

Note: Treatment refers to the presence or absence of an adult predator (hawk) near the nest.
[a] N = 272 observations, 49 nests, 38 females, 34 males.
[b] Males relative to females.
[c] Predator treatment versus baseline.
[d] N = 102 observations, 25 nests, 23 females, 23 males.

Relative male contribution to provisioning declined later in the nestling period, as reflected by a negative interaction between sex and day in stage (Table 4.2), and males contributed relatively more to provisioning larger broods, as reflected by a positive interaction between brood size and sex (Table 4.2). There was no interaction between treatment (predator presence) and sex (LMM: $F_{1,217} = 0.02$, $\beta = -0.01 \pm 0.11$, $P = 0.89$, Figure 4.4a).

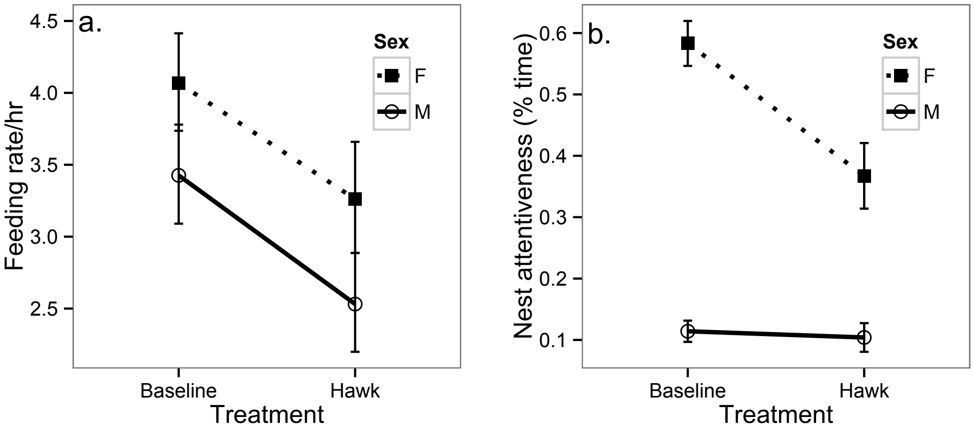

Figure 4.4. Effect of perceived adult-directed predation risk on provisioning (feeding) rates and nest attentiveness in females and males. Females displayed higher nestling provisioning rates (a) and nest attentiveness (b) than males. Both males and females similarly decreased provisioning rates in response to the hawk (a). Only females decreased nest attentiveness in response to the hawk, but male attentiveness was already low under baseline conditions (b). Values represent means and bars denote standard error. Back transformations were applies in the case of feeding rates (a).

Females also displayed higher nestling stage nest attentiveness than males (Table 4.2, Figure 4.4b), and nest attentiveness declined with nestling age (day in stage) and predator presence (Table 4.2). Moreover, there was a positive interaction between sex and treatment (predator presence) in predicting attentiveness, which reflected the fact that females decreased attentiveness given predator presence, while males did not (Table 4.2, Figure 4.4b). Brood size had no effect on nest attentiveness (LMM: $F_{1,21} = 0.01$, $\beta = -0.003 \pm 0.03$, $P = 0.92$), and brood size and nestling age did not interact with sex to predict nest attentiveness (LMM: brood size × sex interaction term, $F_{1,72} = 0.10$, $\beta = 0.01 \pm 0.04$, $P = 0.76$; nestling age × sex interaction term, $F_{1,72} = 3.18$, $\beta = 0.08 \pm 0.05$, $P = 0.08$).

DISCUSSION

Our results are consistent with theory and past empirical evidence suggesting that acute and delta CORT levels are lower in the sex that provides the majority of parental care, such that reduced stress hormone levels accompany higher parental effort (Wingfield et al. 1982, 1995; Wingfield and Sapolsky 2003). Specifically, females, who must produce costly eggs, often replace expensive clutches repeatedly following predation events, are solely responsible for incubation, and have higher certainty of parentage (Trivers 1972; Montgomerie and Weatherhead 1988; Arcese et al. 2002), had smaller stress responses than males. In addition, both sexes down-regulated the stress response during the parentally demanding nestling period, when brood value is high and investing in offspring may be especially crucial to fitness (Bókony et al. 2009). However, the stress response remained higher in males. Males may retain higher stress responses, despite contributing to parental care, if they have less invested in the social brood than females (Trivers 1972; Montgomerie and Weatherhead 1988; Ketterson and Nolan 1994).

Further, statistical analyses performed within each sex separately suggested sex-specific differences in modulation of the stress response with respect to nesting stage and date of capture, which may reflect sex-specific differences in reproductive strategies (Wingfield et al. 1995; Bókony et al. 2009). Specifically, capture stage predicted acute CORT levels and tended to predict delta CORT in males, with levels being lower during the nestling stage. In contrast, in females, delta and acute CORT were not predicted by capture stage, but declined with capture date. Males participate in parental care only during the nestling stage. Thus, the nestling stage transition to paternal care in males may result in a greater difference between early and nestling stage CORT levels in males relative to in females (Wingfield et al. 1995; O’Reilly and Wingfield 2001; Holberton and Wingfield 2003). In contrast, females also invest parentally during incubation. Thus, rather than being predicted by nesting stage, female stress responses may decline with date, as time to re-nest decreases and brood value increases (Bókony et al. 2009).

Like acute and delta CORT, baseline CORT concentrations were lower in females, suggesting that elevated baseline CORT may also act either directly or indirectly to inhibit parental activity. Indeed, past research has supported this contingency. For example, in female tree swallows (*Tachycineta bicolor*) early-season baseline CORT is negatively correlated with egg mass, suggesting that elevated baseline CORT suppresses reproductive investment (Bonier et al. 2009b). Moreover, in the polymorphic white-crowned sparrow, experimentally elevating baseline CORT concentrations in more parental tan-striped males reduces nestling provisioning rates to levels comparable to those found in less parental white-striped males, which normally have higher baseline CORT (Horton and Holberton 2009). However, in our study, baseline CORT did not change with either capture date or nesting stage, which are associated with increased brood value and increased parental duties (Bókony et al. 2009), respectively. Thus, our results suggest that intersexual differences in baseline CORT may not be directly related to parental demands. Parental duties are not the only component of daily energy balance, such that the relationship between hormone levels and parental effort may be complex and indirect (Landys et al. 2006). For instance, higher baseline CORT levels in males, especially early in the season, could reflect high energetic demands of territory establishment and defense and may not directly mediate lower parental investment.

In addition to lower stress responses in females, we also found that females display higher parental effort and risk-taking during the period of biparental care. Since CORT levels were lower in females even during the nestling period, this result is consistent with the idea that modulation of the stress response may help mediate parental decision-making, although we have no way to establish a direct mechanistic link between parental behavior and the HPA axis

(Wingfield et al. 1995; O'Reilly and Wingfield 2001; Holberton and Wingfield 2003). Specifically, we found that females, display both higher offspring provisioning rates and higher nest attentiveness. Despite higher baseline parental effort in females, both sexes similarly decreased offspring provisioning rates in response to adult predator presence. Nevertheless, due to parallel slopes of reaction norms, females maintained higher provisioning rates than males in the presence of the adult predator, suggesting that females accept higher survival costs to maintain offspring wellbeing (Ghalambor and Martin 2001, 2002). Females actually decreased nest attentiveness more than males in response to the predator. However, steeper declines in nest attentiveness in females were an artifact of low average baseline attentiveness in males. Thus, despite decreasing attentiveness more than males in response to adult predation risk, females were still significantly more attentive in the presence of the predator, and presumably subject to higher survival costs. Consistent with our findings, Montgomerie and Weatherhead (1988) report that female *M. melodia* defend nests more vigorously than males, thus placing themselves at greater risk of injury. Therefore, sex differences in CORT levels, baseline reproductive effort and responses to the predator all suggest that females value brood survival more than males.

However, the magnitude of difference between male and female contribution to parental care was modified by brood size and nestling age, suggesting that life-history decisions within each sexes are additionally adjusted based on context-dependent selective pressures. Specifically, given lower brood size males fed nestlings at lower rates than females, whereas sex differences in feeding disappeared at higher brood size. This effect may arise if males up-regulate feeding to match female effort only in the context of larger, more valuable broods that would be difficult for females to rear independently (Ardia 2007). Indeed, past studies have also shown that male contribution to parental care may increase with brood size (Grundel 1987; Moreno et al. 1995). Given equalization of male and female feeding contribution at higher brood size, it would be instructive to examine whether CORT levels within the sexes also vary with brood size. Indeed, in house sparrows (*Passer domesticus*) birds with experimentally enlarged broods display reduced delta CORT relative to controls, although sex-specific modulation with respect to brood enlargement was not explored (Lendvai et al. 2007). In addition, female and male feeding rates were similar when nestlings were young and feeding rates relatively low, while females contributed more to provisioning older nestlings. Females may spend more time brooding, rather than provisioning, young nestlings, but may up-regulate feeding effort when nestling thermoregulatory capacity improves (Arcese et al. 2002; Barg et al. 2006).

In contrast to our population, in which males and females display differences in the stress response and parental effort even during the nestling stage, in other environments maximal contribution to parental care by both sexes may be crucial to nesting success. In those cases, females and males may show equivalent stress responses and parental effort during breeding, as found in northern populations of yellow warblers (Wilson and Holberton 2004) and willow warblers (*Phylloscopus trochilus*) (Silverin et al. 1997). In addition, sex differences in the stress response may exist early in the season, but equalize during the nestling stage if both sexes contribute equally to nestling provisioning, as found both in arctic-breeding white-crowned sparrows and in American tree sparrows (*Spizella arborea*) (Holberton and Wingfield 2003). In a population in which sex differences in CORT levels do not exist during the nestling stage (Holberton and Wingfield 2003), we would predict that males and females contribute equally to parental care and respond similarly to predation risk, reflecting similar balance of the survival-reproduction tradeoff between the sexes.

Finally, our results were consistent with the prediction that body condition declines over the course of the breeding season, which could be interpreted as reflecting self-maintenance costs associated with reproduction (Ricklefs 1974; Askenmo 1977). However, female body condition was not lower than male condition during the breeding season, as might be expected given higher reproductive investment. Rather, males were in poorer condition than females early in the season, and condition was not predicted by sex during the nestling stage. Thus, female condition declined more between the early season and the nestling stage. Sharper declines in body condition in females could be interpreted as supporting the prediction that higher female parental investment results in greater self-maintenance costs, and could result from costs of incubation or higher female provisioning rates (Ricklefs 1974; Askenmo 1977; Bryant 1979). However, rather than reflecting differences in reproductive effort, sex differences in body condition change may also reflect distinct reproductive strategies and dynamic balance of the tradeoff between maintaining energy reserves and maximizing flight efficiency to reduce predation risk. Specifically, females may gain weight early in the season in preparation for energetically costly investment into eggs and prolonged bouts of incubation, but may reduce mass later in the season to increase flight efficiency (Freed 1981; Cichon 2001; Suarez et al. 2005). Males face high energetic demands of territory establishment early in the season, which may lead to lower early-season condition. However, males also do not participate in egg production or incubation, and thus may not benefit from amassing early season energy stores (Merkle and Barclay 1996; Geslin et al. 2004). Indeed, when compared to unsupplemented controls, mountain bluebirds (*Sialia currucoides*) provided with supplementary food displayed similar patterns of body mass modulation over the course of the breeding season, supporting the flight adaptation hypothesis, especially for females (Merkle and Barclay 1996).

CONCLUSIONS

Our study yields several important conclusions. Firstly, lower CORT levels in females, the more parental sex, and lower stress responses in both sexes during the parentally demanding nestling stage, support theory suggesting that the stress response should negatively covary with parental effort and brood value (Romero 2002; Wingfield and Sapolsky 2003; Bókony et al. 2009). Secondly, despite being lower in females, baseline CORT did not vary within either sex as a function of nesting stage or capture date, suggesting that regulation of baseline CORT is not closely linked to regulation of parental activity in our species (Landys et al. 2006; Bókony et al. 2009). Thirdly, our behavioral data confirm that females, which had lower stress responses during the biparental nestling stage, displayed higher parental effort and risk-taking for nestlings than males. These results suggest that the HPA axis may indeed mediate parental decision-making processes that affect adaptive balance of the survival-reproduction tradeoff, although the nature of our data make establishing a mechanistic link between CORT levels and parental effort impossible. Future research should explore whether populations without sex differences in nestling stage CORT levels display equal reproductive effort and risk-taking behavior, and whether sex differences in CORT levels and parental behavior are modified as a function of other factors that affect brood value such as brood size, nestling age, and cues indicative of offspring fitness.

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CHAPTER 5: Phenotypic plasticity in nest departure calls: weighing costs and benefits

ABSTRACT

In birds male song has been extensively studied, but female vocalizations have received little attention. Females of several North American species produce a unique vocalization, the nest departure call (NDC), upon leaving nests. Producing NDCs has costs due to acoustical properties that make nests easy to locate by predators. Thus, NDCs must also have benefits that balance or outweigh costs, and females should modulate call production as costs and benefits change. We explored whether female song sparrows (*Melospiza melodia*) adjust calling rate to reflect differential costs and benefits of calling induced by male presence, male quality (measured by body condition and song complexity), nest predator presence, and nest height. Results suggest that calls benefit females by promoting male nest guarding and that females display adaptive plasticity in call production. Specifically, calling rate increased when the male was present, and male nest guarding increased when females gave a NDC. Females called less in the presence of a model nest predator, likely because perceived costs of predator attraction outweighed benefits of male recruitment. Conversely, females with mates in good condition called more, perhaps because the efficacy of male nest guarding increases with condition. In addition, females called more from elevated nests in the presence of the predator, and decreased calling later in the day. Male song complexity failed to predict calling rate, suggesting that this sexually selected trait does not reflect direct benefits gained by producing a NDC. Plasticity in calling likely exists because context-appropriate communication elevates fitness, whereas contextual mistakes in the decision to communicate result in fitness declines. *Keywords*: Behavioral plasticity, nest departure calls, nest guarding, cost-benefit analysis, communication, *Melospiza melodia*

INTRODUCTION

The complex and prominent vocalizations of male songbirds are the focus of extensive study (Searcy and Andersson 1986; Nowicki and Searcy 2004). However, the subtler vocalizations of females have received little attention (McDonald and Greenberg 1991; Gorissen and Eens 2005). One unique type of female vocalization that has been recorded in at least 15 species of North American passerines is the nest departure call (NDC). NDCs are initiated upon leaving the nest, and have a characteristic acoustical structure including broad-band frequency, short note duration, and repetitiveness, that makes nests easy to localize, and also acts to project flight trajectory (McDonald and Greenberg 1991; Figure 5.1).

Passerine nests are vulnerable to predation, and nest depredation has played a pivotal role in driving the evolution of species-level differences in incubation behavior (Ricklefs 1969; Martin 1995; Conway and Martin 2000; Martin et al. 2000). Thus, the adaptive function of highly conspicuous calls, given from nests and open to exploitation by predators, evades easy explanation. Indeed, empirical research has demonstrated that giving NDCs can increase nest predation rates (Yasukawa 1989; McDonald and Greenberg 1991), as well as parasitism by brown-headed cowbirds (*Molothrus ater*) (Clotfelter 1998). Thus, NDCs must have benefits that override these costs. Previously hypothesized benefits of calling include recruiting male vigilance for nest guarding during female absence (Yasukawa 1989; McDonald and Greenberg 1991), decreasing harassment of females by males that may mistake them for territorial intruders (Beletsky and Orians 1985; Edwards 1987; McDonald and Greenberg 1991), advertising female receptivity, discouraging settlement of other females on the territory, distracting predators (McDonald and Greenberg 1991) and recruiting males to mate-guard during incubation off-bouts (McDonald and Greenberg 1991; Fedy and Martin 2009).

However, due to the paucity of work on this behavior, the actual functions of calls and associated costs and benefits remain poorly understood (McDonald and Greenberg 1991). Moreover, communication systems have evolved to elevate fitness, but signaling in the wrong context may result in fitness declines (Zuk et al. 1995; Zuk and Kolluru 1998). Thus, individuals should exhibit phenotypic plasticity in calling behavior (Stearns 1989; Lima and Dill 1990; McNamara and Houston 1996). However, no study has comprehensively investigated whether females adjust calling behavior in a context-dependent fashion to maximize benefits and minimize costs. We address these gaps in understanding by addressing novel questions about the function and context-dependency of NDCs in female song sparrows (*Melospiza melodia*). Our study had five primary objectives, which we outline below.

Firstly, we explored whether females plastically adjust NDC production in a fashion consistent with the hypothesis that calls function as a signal to increase male vigilance during incubation off-bouts. Given a male-recruitment function, benefits of calling should be high when the male is near, but calling should have little adaptive benefit in absence of the male, such that calling rate increases in the presence of the male. Further, the benefit of calling should be manifest by an increase in male nest-guarding behavior during incubation off-bouts following a NDC.

Secondly, we sought to determine whether the presence of a nest predator affects female call production. Avian nest predators have highly developed auditory systems and may thus use NDCs to locate nests (Martin 1987a; Eggers et al. 2006; Peluc et al. 2008). Thus, we predicted that females would reduce call production in the presence of perceived corvid nest predation risk as an adaptive means of reducing the likelihood of nest predation.

Thirdly, we examined whether male quality modifies female calling behavior. Specifically, we considered effects of two indicators of male quality on NDC production: body condition and song complexity. Males in good body condition have high energy reserves, may devote less time to foraging and self-maintenance, and may thus be more effective at nest defense (Wallin 1987; Winkler 1992; Martin and Horn 1993; Sproat and Ritchison). Additionally, good condition may reflect high territory quality, which may increase both foraging efficiency and time available for vigilance (Drent and Dann 1980; Martin 1987b; Komdeur 1992; Svensson and Nilsson 1995; Van de Crommenaker et al. 2011). Thus, we predicted that benefits of calling and NDC production increase with male body condition. Song complexity is the basis for female choice in many species, including *M. melodia* (Searcy 1984; Searcy and Andersson 1986; Searcy and Yasukawa 1996; Nolan and Hill 2004; Nowicki and Searcy 2004), and female choice of males with complex songs may be motivated by direct benefits gained in the form of paternal assistance. Thus, we predicted that benefits of calling and NDC production would increase with male song complexity (Hoelzer 1989; Hill et al. 1991; Buchanan and Catchpole 2000). However, an alternative hypothesis is that females choose males with complex songs primarily for indirect benefits, and males with more complex songs tradeoff mating effort against paternal effort (Burley 1988; Møller and Thornhill 1998; Qvarnström et al. 2000). In this case, nest guarding services and NDC production might decline with song complexity. Further, with respective to male quality in general, we predicted that female call production would decline less in the presence of the predator when the male was of higher quality, since benefits of male recruitment and guarding might offset costs of predator attraction in this case.

Fourthly, we explored the effect of nest site location on calling behavior (Martin 1987a; Martin 1995; Martin et al. 2000; Eggers et al. 2006; Peluc et al. 2008). More concealed nests may be more difficult to locate, lowering costs of calling. Indeed, across avian taxa, NDCs are given almost exclusively by species occupying marshland or grassland habitat, where dense cover may provide a buffer against nest detection (McDonald and Greenberg 1991). Further among open-cup passerines, ground nests are subject to the lowest predation rates, with nests elevated in the canopy or shrubs experiencing higher depredation (Martin 1993, 1995; Peluc et al. 2008). In song sparrows at our study site, nests on the ground tend to be more concealed than elevated nests (Authors, personal observation). Moreover, elevated nests are more exposed to visually oriented corvid predators (Martin 1987a; Peluc et al. 2008). Thus, we used nest placement on or off the ground as a proxy for nest concealment. We predicted that costs of calling at elevated nests would outweigh benefits, resulting in higher calling rates at on-ground nests, and that this effect would be magnified in the presence of the predator.

Finally, our fifth objective was to establish whether NDC production covaries with other aspects of incubation behavior. Specifically, we were interested in whether recruitment of the male via NDCs allows females to spend more time off the nest engaging in self-maintenance activities, such that off-bout length increases and overall nest attentiveness decreases as a function of giving the call. By establishing whether male-female communication via the NDC affects optimal patterns of incubation, we hoped to grant insight into the under-explored contingency that interactions between mated partners modify patterns of parental effort in biparental species (Fedy and Martin 2009).

METHODS

Study species and site

We studied NDCs in song sparrows (*Melospiza melodia*) breeding near the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL) on the eastern slope of the Sierra Nevada (Mono County, CA, 37º36'51"N/118º49'47"W). Focal pairs occupied territories along the riparian corridors of Convict and McGee Creek. Convict Creek flows through SNARL. McGee Creek is located ~10 miles south of SNARL in the Inyo National Forest (37°33'20"N/118°47'35"W). Both sites are at mid-elevation (2,100-2,500 meters). Interestingly, McDonald and Greenberg (1991) report that *M. melodia* females generally do not produce NDCs. However, females from Mandarte Island and around Puget Sound do produce the calls (McDonald and Greenberg 1991), as do females in our study population.

In *M. melodia* the female alone incubates, whereas both females and males provision nestlings. However, males may contribute to parental care during incubation via nest guarding. Nest guarding may have a particularly potent impact on fitness during incubation off-bouts, when the female leaves the nest to forage and engage in other self-maintenance activities. In our song sparrow population the mean ± SE incubation on-bout is 19.96 ± 1.08 minutes (range 7.39 - 48.44 minutes), and the mean off-bout length is 7.68 ± 0.49 minutes (range 2.02 - 24.25 minutes). Male singing activity often increases near the nest during incubation off-bouts of females (Authors, personal observation).

High nest predation rates in our population (68.13%) may increase the selective importance of NDCs and male nest guarding. We confirmed the identity of few predators. However, long-tailed weasels (*Mustela erminea*) and garter snakes (*Thamnophis* sp.) were seen removing nestlings. Further, nests also faced high parasitism by brown-headed cowbirds (*Molothrus ater*) (28.9%). Corvids, including western scrub-jays (*Aphelocoma californica*), Stellar's jays (*Cyanocitta stelleri*), and black-billed maypies (*Pica hudsonia*), and small mammals, including least chipmunk (*Neotamias minimus*) and deer mice (*Peromyscus maniculatus*) also contribute to predation on open-cup passerine nests in eastern Sierra riparian corridors (Latif et al. 2012).

Research approach

Beginning in early May 2010 to 2012 we located and target netted breeding pairs of *M. melodia* as part of a larger study on nesting behavior. We used conspecific playback to expediently lure males into mist nets. We uniquely banded birds with USGS bands and an additional combination of three colored leg bands. The breeding season extended through mid-August.

Field techniques were authorized by a USGS bird banding permit (23035-F), a California state collecting permit (SC-11059), a federal migratory bird collecting permit (MB22670A-0) and a special use permit from the Inyo National Forest (MLD100008P). The University's Institutional Animal Care and Use Committee (protocol A-20100002E) approved all animal use procedures.

Behavioral observation and predator presentation experiment

We located nests using a combination of systematic search and behavioral observation (Martin and Geupel 1993). Once nests were located, we checked contents every 2 to 3 days to document nesting status. On day 2 to 8 of the incubation period, or as soon as the nest was located, we video-recorded nests using Canon 800 series camcorders under three experimental conditions: baseline, elevated perceived nest predation risk, and negative control. We elevated perceived nest predation risk using a decoy and recording of a Western scrub-jay (*Aphelocoma californica*) (Peluc et al. 2008). A model and recording of a house finch (*Haemorhous mexicanus*) served as a negative control. We placed decoys 6 to 10 m from nests and 2 m off the ground, and projected recordings of vocalizations using a MP3 player placed on the ground below decoys. We concealed camcorders 3 to 6 m from nests to prevent disturbance originating from the recording alone. Presentations were initiated between 0600 and 1400 PDT. We controlled for time of day and date in statistical analyses. We performed treatments sequentially in randomly assigned order. Each treatment lasted approximately 2 hours. We measured nest height after the nest failed or fledged.

Data extraction from video-recordings

We extracted NDC behavior for a random subset of nests recorded. We previously established that female song sparrows respond to scrub-jays as predators by reducing incubation attentiveness relative to under baseline conditions, but do not alter incubation attentiveness in response to the house finch (M. Grunst, unpublished data; see Appendix 2, Table 2.A1). Further, preliminary analysis indicated that females did not reduce calling behavior in response to the finch, as would be expected if the finch was perceived as disturbance or if the noise of the recording alone interfered with communication (generalized linear mixed effects model: $Z = 1.45$, $\beta = 1.13 \pm 0.78$, $P = 0.14$). Thus, we only extracted data on NDC behavior from 10 finch recordings (the process is time intensive), and coded finch treatments as baseline for use in this analysis. We analyzed NDC behavior from a sample including 427 nest departures, 61 nests, 41 males, and 47 females. We extracted NDC data for both the predator and baseline treatments for 39 nests (336 nest departures, 30 males, 32 females), for baseline alone for 8 nests (33 nest departures, 8 males, 8 females), and for the predator alone for 14 nests (58 nest departures, 16 males, 13 females). Results were qualitatively the same when restricting the dataset to the subset of nests for which we conducted both baseline and predator trials. The sample size for the final model predicting NDC production is reduced since we only measured the body condition of 35 males. Further, the model used to test for covariation between NDC production and off-bout length excluded some nest departures for which the off-bout length was unknown due to the recording ending before the female returned. In addition, we only measured the repertoire size of 36 males and the syllable diversity of 35 males.

We viewed recordings to determine when females departed the nest and whether a NDC was given upon each departure. Additionally, to determine whether the male was present before female departure from the nest, we recorded whether the male produced a song during the 3 minutes immediately prior to departure. If a song was produced during this period, or the male was seen, the male was considered present. If not, he was considered absent (coded 1, 0). As a metric of male nest guarding we recorded whether the male produced a song in the 3 minutes following female nest departure. If a song was produced during this period, or the male was seen, the male was considered present, if not he was considered absent (coded 1, 0). This procedure for determining male presence could be prone to producing false negatives, since males might be present, but not vocal. False negatives could reduce our ability to detect a correlation between male presence and female calls, but should not otherwise affect the validity of statistical tests.

Measuring body condition and song complexity

Upon capture of males, we measured body mass (± 0.1 g) using a digital scale and unflattened wingchord (± 1 cm) using a wing scale. We subsequently calculated body condition using residuals of a regression of body mass on wingchord (Albretch et al. 2005).

To obtain song of focal males, we used iMovie and Quicktime to extract .wav audio files from mini DVD tapes containing video-recordings of incubation behavior. We visualized 300 consecutive songs or 450 total songs per male using *Raven Pro* (Cornell Lab of Ornithology) (Pfaff et al. 2007; MacDougall-Shackleton et al. 2009). We measured two metrics of male song complexity: song repertoire size and song syllable diversity. We determined distinct song types upon song type switching in strings of vocalizations. Once repertoire size was established, we counted syllable number for each song type (MacDougall-Shackleton et al. 2009).

Statistical analysis

We used R 2.15.2 to conduct all statistical analyses (R Core Team, 2012). To assess effects of male presence, predation risk, male quality, and nest height on the decision to produce a NDC, we used a generalized linear mixed effects model (GLMM, binomial family). The dependent variable was whether or not the female produced a call upon leaving the nest (coded 1, 0). We included treatment (baseline, predator), male presence (coded 1, 0), body condition, repertoire size, syllable diversity, and nest site elevation (0 = on ground, 1 = off ground) in the model as fixed effects. We initially allowed for two-way interactions between treatment and all other independent variables, since we were interested in how costs associated with predator presence would modify relationships. We also included time and date in the model as covariates. We entered nest number, male identity and female identity as random effects.

Secondly, to assess the effect of NDC production, predator presence, and male phenotype on male recruitment to guard the nest we again used a GLMM binomial model with guarding (coded 1, 0) as the dependent variable. We included treatment, NDC (coded 1, 0), body condition, repertoire size, and syllable diversity in the model as fixed effects, and used the same interaction and random terms as described above.

Thirdly, to test for covariation between NDCs and incubation behavior, we used a linear mixed effects model (LMM) fit using reduced maximum likelihood, with the length of the off-bout immediately following each nest departure entered as the dependent variable. We entered treatment, NDC (coded 1, 0), male body condition, repertoire size, and syllable diversity as fixed effects, and used the same interaction terms, random terms and covariates as described above. We transformed off-bout length by taking the cube root. We then used a linear mixed effects model to examine whether overall nest attentiveness (percent time incubating eggs) was influenced by the percentage of time that the female gave a NDC, predator presence, and male characteristics. We squared incubation attentiveness to achieve normality. We did not use interaction terms in this model, but again included the same random terms and covariates described above. We employed a Satterthwaite approximation (implemented by the lmerTest package of R) for estimating degrees of freedom in LMM models. We sequentially reduced all models until remaining predictors were significant ($\alpha = 0.05$).

RESULTS

Characterization of calls

Song sparrow NDCs displayed the broad-band frequency, short note duration, and repetitiveness characteristic of NDCs described previously (Figure 5.1). Females produced calls upon initiation of departure from the nest, often shortly after the male sang close by (Figure 5.2). Females produced NDCs at 33.72 % of nest departures during incubation.

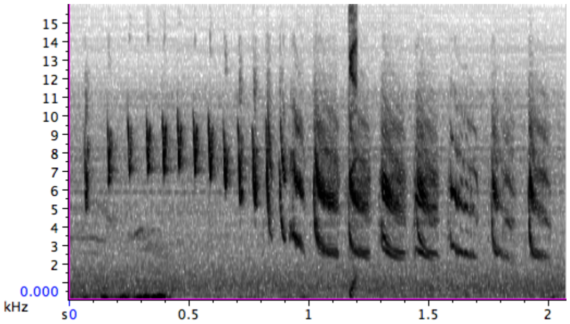

Figure 5.1. Sonogram of a nest departure call exhibiting characteristic broad-band frequency, short note duration, and repetitiveness.

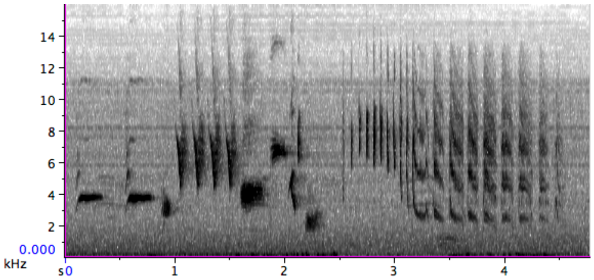

Figure 5.2. Sonogram of a male song followed by a nest departure call. Nest departure calls, and ultimate nest departure, are often associated with the male singing in close proximity to the nest.

Nest departure call production

Male presence was the strongest predictor of the decision to produce a NDC (Table 5.1). When the male was present, females produced calls at 42.75 % of nest departures, whereas in the absence of the male the females called at only 17.22 % of departures (Figure 5.3a). In addition, females produced fewer NDCs in the presence of the scrub-jay nest predator (Table 5.1). Specifically, females produced NDCs at 38.76 % of departures under baseline conditions, but at only 28.90 % of departures in the presence of simulated predation risk (Figure 5.4). Females called more when their mate was in better body condition, and less later in the day (Table 5.1). Finally, there was a positive interaction between nest height and treatment, which reflected the fact that females produced the call more often in the presence of the predator when departing from off-ground nests (Table 5.1). No other interactions were significant (GLMM: $P > 0.1$ in all cases; Appendix 5, Table 5.A1) and female calling did not differ as a function of male repertoire size or syllable diversity (GLMM: $P > 0.1$; Appendix 5, Table 5.A1).

Table 5.1. Generalized linear mixed effects model (binomial family) predicting NDC production by female *M. melodia*

	Estimate ($\beta \pm$ SE)	Z	P (> | z |)
Male present	2.49 ± 0.52	4.79	< 0.001
Treatment	-2.98 ± 1.05[a]	-2.83	0.005
Male condition	1.60 ± 0.47	3.41	0.001
Nest height	-0.13 ± 0.95	-0.14	0.89
Time of day	-0.37 ± 0.13	-2.87	0.004
Treatment x nest height	3.02 ± 1.15	2.63	0.009

[a] Predator (jay) treatment relative to baseline.

N = 358 nest departures, 52 nests, 42 females, and 35 males.

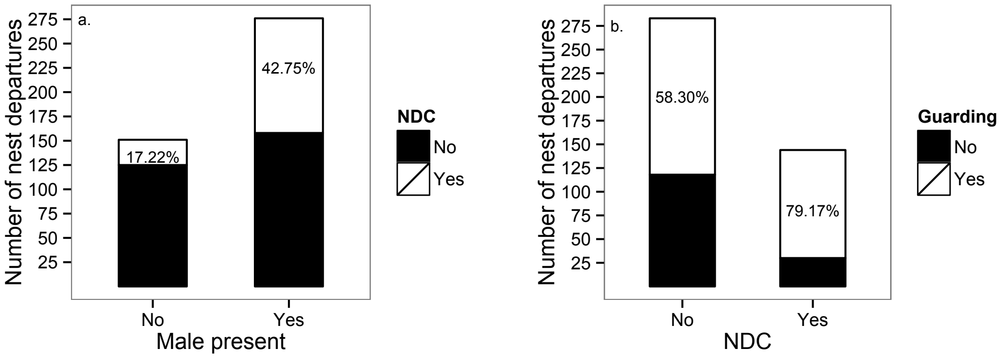

Figure 5.3. Relationships between male presence and production of a nest departure call (a) and the production of a nest departure call and male nest guarding (b). Frequency of NDCs increased significantly in the presence of the male (a), and when a call was given males were more likely to nest guard (b).

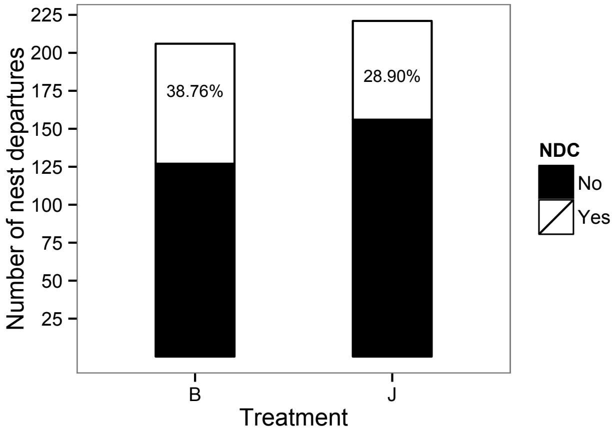

Figure 5.4. Effect of nest predator presence on the production of a nest departure call. Frequency of NDCs decreased in the presence of elevated perceived corvid nest predation risk. Treatment codes: B = baseline, J = jay.

Male nest guarding

Following female nest departure, males were significantly more likely to guard the nest after a NDC was given than if the female produced no call (GLMM: $Z = 3.16$, $P = 0.002$, $N = 427$ nest departures, 61 nests, 47 females, 41 males). Specifically, males recruited to guard the nest during 79.17 % of off-bouts following a NDC, whereas when the female did not call, males recruited during 58.38 % of off-bouts (Figure 5.3b). This pattern did not depend on the presence of the nest predator, as there was no main effect of predator presence and no interaction between NDCs and the presence of a predator ($P > 0.1$ in all cases; Appendix 5, Table 5.A2). Male body condition, song complexity, and nest height also failed to predict guarding behavior, and there was no indication of additional interactions ($P > 0.1$ in all cases; Appendix 5, Table 5.A2). Model results were qualitatively the same when we restricted the analysis to observations in which the male was present before nest departure.

Covariation with incubation behavior

The length of independent off-bouts was not significantly related to whether the female gave a NDC, nest predator presence, or male characteristics, although there was a weak trend towards off-bout length positively covarying with NDC production (GLMM: $P > 0.05$ in all cases; Appendix 5, Table 5.A3). The only significant predictor of off-bout length was time of day, with off-bout length increasing later in the day (LMM: $F_{1, 212} = 4.44$, $P = 0.04$, $N = 395$ nest departures, 61 nests, 47 females, 41 males). Total nest attentiveness (percent time spent incubating eggs) was similarly unrelated to the percentage of time that a female gave a departure call, male song characteristics or predator presence (GLMM: $P > 0.1$ in all cases; Appendix 5, Table 5.A4). However, females were more attentive when mated to males in higher body condition (LMM: $F_{1, 43} = 4.23$, $P = 0.04$, $N = 84$ observations, 45 nests, 35 females, 31 males). Significant and non-significant results for models predicting NDC production, nest guarding and incubation behavior are summarized in Table 5.2.

Table 5.2. Summary of main effects for NDC frequency, male guarding behavior and incubation attentiveness/off-bout length

Dependent variable	Independent variable	Direction of effect	P
Nest departure call behavior	Male presence	+	<0.001
	Nest predator presence	–	0.005
	Male condition	+	0.001
	Male song complexity	0	>0.1
	Nest height	0	>0.1
Male guarding behavior	Nest departure call given	+	<0.001
	Nest predator presence	0	>0.1
	Male condition	0	>0.1
	Male song complexity	0	>0.1
	Nest height	0	>0.1
Incubation attentiveness/off-bout length	Nest departure call given	0 / trend +	>0.1 / 0.08
	Nest predator presence	0	>0.1
	Male condition	+ / 0	0.04 / >0.10
	Male song complexity	0	>0.1

DISCUSSION

Our results are consistent with the hypothesis that NDCs given by female song sparrows function to recruit male vigilance during incubation off-bouts. Females called more often when the male was present before nest departure, and males were also more likely to nest guard when the female gave the call. The increase in calling rate in the presence of the male does not necessarily refute the alternative hypothesis that calls act to decrease male harassment of the female (Beletsky and Orians 1985; Edwards 1987; McDonald and Greenberg 1991). Indeed, in red-winged (*Agelaiusp hoeniceus*) and yellow-headed (*Xanthocephalus xanthocephalus*) blackbirds, males chase females that leave the nest silently significantly more often than females that vocalize upon nest departure. Thus, calls in these species may function to decrease harassment, which might otherwise distract females from adaptive incubation behavior (Beletsky and Orians 1985; Edwards 1987). Since we have no data on chasing behavior in song sparrows, we cannot discount the possibility that silent departures result in harassment. However, our results do suggest that calls do not function to increase male mate guarding behavior (Fedy and

Martin 2009). Specifically, males more often remained in the vicinity of the nest after a NDC, whereas females usually left the vicinity to forage. Given that NDCs function as signals to enhance male nest guarding, calling behavior may evolve in populations or species in which nest guarding by males is especially important to fitness.

In populations with high rates of nest predation, producing NDCs to promote male nest guarding may have high fitness benefits. On the other hand, given characteristics of the NDC that make the vocalizer easy to locate, producing the call may attract predators (McDonald and Greenberg 1991). Thus, females may elevate fitness by plastically adjusting calling behavior to target the intended receiver (the male), while avoiding eaves dropping by predators (Zuk et al. 1995). Indeed, calling rate declined when we experimentally elevated perceived nest predation risk. Like other avian nest predators, scrub-jays have highly developed auditory systems, and may cue in to NDCs when depredating nests (Martin 1987a; Eggers et al. 2006; Peluc et al. 2008). Significantly, when not producing a NDC, females often departed from the nest silently, and commenced alarm calling once off the nest (Authors, personal observation). In contrast to NDCs, acoustical characteristics of alarm calls, including high frequency and short duration, make localization difficult (Caro 2005; Klump and Shalter 2010). Thus, switching from the NDC to the alarm call in the context of high nest predation risk could provide a mechanism of alerting the male to nest departure while reducing the probability of nest detection.

In addition to predator presence, differences in the dominant predator type might also affect the adaptive advantage of producing NDCs. In contrast to avian predators, other predator guilds such as snakes have poor hearing, are unlikely to use calls to locate nests, and may selectively depredate lower nests as opposed to higher ones (Martin 1987a; Peluc et al. 2008). Further, some types of predators are easier to defend against, such that intensity and efficacy of nest defense may vary with predator type (Kruuk 1964; Curio 1975; Patterson et al. 1980; Winkler 1992). If a predator is easily deterred, benefits of attracting the male might outweigh costs. Thus, an intriguing possibility is that females may actually increase calling rate when faced with predation threat from a different type of predator. We do not know the relative contribution of predator guilds to nest depredation events in our population. However, recent research on the predator community of open-cup nests in eastern Sierra riparian habitat concluded that one or a few predator species do not predominate (Latif et al. 2012). Therefore, females may need to assess a complex predator community when adjusting calling behavior.

Further, females may also need to assess their mate's willingness or ability to nest guard when determining whether to call. Indeed, females were more likely to give a NDC if their mate was in good body condition. Males in better body condition may be more willing or able to defend nests against predators, such that costs of attracting predators decline when the male is in good condition. Studies in a number of bird species have reported a positive correlation between nest guarding intensity and condition (Wallin 1987; Winkler 1992; Martin and Horn 1993; Sproat and Ritchison 1993). Further, in *M. melodia*, Rastogi et al. (2006) demonstrated that food-supplemented birds have more time available for nest guarding and nest attentiveness than unsupplemented controls. Guarding, as measured by male presence during incubation off-bouts, was uncorrelated with male body condition in our study, suggesting that male willingness to defend the nest did not play a dominant role. However, we did not directly measure the intensity of male nest guarding, the tendency for males to take risks to defend the nest, or efficacy of deterring predators. In contrast to body condition, NDC rate was unrelated to male song complexity, suggesting that song may convey little information regarding direct benefits provided by males via nest guarding. Male nest guarding was similarly unrelated to song complexity, corroborating this view.

In addition, females may also adjust calling behavior based on differences in nest placement that affect vulnerability to detection by predators. We hypothesized that elevated nests would be more vulnerable to predation by corvids (Martin 1987a; Eggers et al. 2006; Peluc et al. 2008), such that females with elevated nests should reduce calling rate more when confronted with a predator. In support of this hypothesis, orange-crowned warblers (*Vermivora celata*) nesting in shrubs decreased offspring provisioning rates (another conspicuous parental behavior) more in response to the presence of an avian predator than those nesting on the ground (Peluc et al. 2008). However, we unexpectedly found that females departing from elevated nests actually called more in the presence of the predator. This result may reflect the fact that our study involved production of a conspicuous auditory cue in addition to the visual cue of nest visitation. At elevated nests, predators may be able to use the visual cue of female departure to locate the nest, whereas females may be able to depart from ground nests invisibly. Thus, calling from an on-ground nest may add a more substantial additional cost to departure itself than calling from an off-ground nest. Given the possibility of departure without detection from an on-ground nest, not calling may be adaptive, despite the fact that the male may fail to recruit for nest guarding. On the other hand, departure from an elevated nest without detection may be unlikely, such that it may be adaptive to call, risk predator attraction, but also increase the probability of promoting male vigilance.

Females also called less upon nest departure as the day progressed. The dependency of calling behavior on time may be related to temporal variation in predation risk, male propensity to respond to calls, or female energy balance. Specifically, costs of calling may increase later in the day due to heightened predation risk by diurnal predators. Perplexingly, many predators including brown-headed cowbirds, are more active towards dawn (Rothstein et al. 1984). However, at our mid-elevation study site, mornings are cool and predator activity, especially that of ectothermic predators such as snakes, may be depressed early in the day. Secondly, benefits of calling may decrease later in the day if male propensity to respond to calls declines as the day progresses. However, we did not detect an association between male nest guarding and time of day. Lastly, if calling is energetically expensive, females may call less later in the day as nightfall approaches and foraging time declines (Houston et al. 1997).

Finally, patterns of incubation behavior did not strongly covary with NDC production. Specifically, neither the length of individual off-bouts nor overall incubation attentiveness significantly differed as a function of giving the call. We initially hypothesized that increased male vigilance and decreased costs of staying off the nest associated with producing a NDC would result in longer incubation off-bouts following call production. Similarly, we reasoned that overall nest attentiveness might be lower at nests where females called more often. Consistent with our predictions, there was a trend towards positive covariation between calling and off-bout length in our initial model ($P = 0.06$), suggesting that interactions between males and females associated with NDC production may alter costs associated with time spent off the nest to some extent. However, this trend was very weak in a fully reduced model ($P = 0.13$). Our failure to find a conclusive relationship between NDC production and incubation behavior suggests that the optimal length of incubation off-bouts is not strongly affected by the heightened probability of male recruitment accomplished by giving the call. Indeed, although male nest guarding may lower costs of staying off the nest by reducing the probability of clutch loss through depredation, thermoregulatory costs of remaining off the nest also exist (Webb 1987; Conway and Martin 2000; Martin et al. 2007). Since only females incubate, the presence of the male does not affect thermoregulatory costs. Thus, despite male nest guarding, females may still optimize fitness by minimizing off-bout length to the amount of time needed to accomplish basic self-maintenance activities (Conway and Martin 2000).

CONCLUSIONS

We addressed a novel question regarding the function and context-dependency of NDCs. Results suggest that calls function to recruit males to guard nests. Moreover, females display adaptive plasticity in call production in a fashion consistent with balancing costs and benefits. Plasticity in calling likely exists because context-appropriate communication elevates fitness, whereas contextual mistakes in the decision to communicate result in fitness declines (Tuttle and Ryan 1981; Zuk et al. 1995; Zuk and Kolluru 1998). Thus, our study aids in elucidating the complexity of communication systems in biparental species, and potential fitness ramifications of communicating in inappropriate ways.

More work is needed to fully understand context-dependency in NDCs and potential costs of calling. Examining population-level differences in call production, such as those documented in *M. melodia*, may help elucidate the evolution and persistence of NDCs. In addition, the unique selective pressures of urban environments provide an opportunity to explore impacts of signal interference on call production (Brumm 2004; Bermúdez-Cuamatzin 2009). Finally, learning is central to the production of species-specific song (Beecher and Brenowitz 2005; Catchpole and Slater 2008), but the degree to which learning shapes NDC development, the propensity to produce calls in specific contexts, and persistence of calling within populations is unknown.

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CHAPTER 6: Variation in adrenocortical stress physiology and condition-related metrics within a heterogeneous urban environment

ABSTRACT

In urban habitats organisms face unique fitness challenges including disturbance from human activity and noise. One physiological mechanism that may be plastically or evolutionarily modified to ameliorate deleterious effects of anthropogenic disturbance is the adrenocortical stress response. Individuals in urban environments may display smaller stress responses, which may prevent pathologies associated with consistent elevation of stress hormones, and may also show differences in baseline corticosterone (the primary avian stress hormone), due to altered energetic demands or chronic stress. We examined whether stress physiology and condition metrics in urban song sparrows (*Melospiza melodia*) vary as a function of discrete differences in anthropogenic disturbance level (activity centers and refuges) or with continuous variation in the noise environment. Maximal corticosterone levels after stress did not differ between habitat types, and activity center males showed no evidence of increases in baseline corticosterone, declines in body mass or hematocrit, or changes in antioxidant capacity. Further, males breeding in activity centers tended to display smaller magnitude stress responses (maximal – baseline corticosterone) and higher quality feathers (indicative of higher condition at molt) than activity refuge males. With respect to noise environment, males breeding on noisier territories within activity centers tended to have lower baseline corticosterone concentrations, but did not differ with respect to the acute stress response, body mass, hematocrit or antioxidant capacity when compared to other males. Overall, results suggest that song sparrows are an urban adapter species, which are not detrimentally affected by unique selective pressures encountered in activity centers. *Keywords*: adrenocortical stress response, urbanization, anthropogenic disturbance, habitat quality, physiological adaptation, *Melospiza melodia*

INTRODUCTION

Organisms evolve traits that allow maintenance of positive fitness pay-offs in a particular environment or niche (Grinnell 1917). When faced with rapid fluctuations in the environment species persist through either rapid evolution or phenotypic plasticity. Alternatively, extirpation may result (Coppack and Partecke 2006; Partecke et al. 2006; Ghalambor et al. 2007). Current rapid rates of habitat modification by humans serve as a potent evolutionary force. In urban habitats individuals face fitness challenges including disturbance from loud noises and human activity, pollution from chemicals and artificial light, and altered predator regimes, all of which differ from selective pressures experienced throughout most of a species' evolutionary history (Marzluff 1997; Marzluff 2001; Sih et al. 2011; Fischer et al. 2012; Sol et al. 2013). Thus, in urban areas only species or individuals with a particular suite of ecological and physiological traits may persist (Yeh and Price 2004; McGlothlin and Ketterson 2008; Atwell et al. 2012; Bonier 2012).

One physiological mechanism that may be evolutionarily or plastically modified to ameliorate deleterious effects of frequent urban disturbance is the adrenocortical stress response (Partecke et al. 2006; Bonier et al. 2007; Bonier 2012). The adrenocortical stress response is controlled by the hypothalamus-pituitary-adrenal (HPA) axis and involves release of steroid glucocorticoids (GCs) from the adrenal cortex. Baseline GCs help regulate glucose metabolism and energy balance, and may be elevated to support demanding life-history stages (Sapolsky et al. 2000; Landys et al. 2006). Thus, in urban environments, increased baseline GCs may result from increased allostatic challenge (the summation of current and predicted energetic demands) and may be adaptive.

However, frequent disturbance in urban areas may overwhelm internal coping mechanisms, leading to allostatic overload, chronic elevation of baseline GCs and reduced fitness (McEwen and Wingfield 2003; Wingfield 2005; Bonier 2012). Indeed, elevated baseline GCs have been linked to reduced body condition and survival probability (Romero and Wikelski 2001; Brown et al. 2005), increased oxidative stress due to heightened metabolism (Lin et al. 2004), and cessation of reproductive activity (Silverin 1986; Wingfield and Sapolsky 2003; Ouyang et al. 2012). Activation of the acute stress response controls the vertebrate response to life-threatening situations, and clearly has fitness benefits (Sapolsky et al. 2000; Breuner et al. 2008). However, frequent activation of the acute stress response may lead to termination of reproductive activity and health problems associated with elevated GCs (Wingfield et al. 1992; Sapolsky et al. 2000; Wingfield and Sapolsky 2003). In urban environments many human-associated disturbances may entail little actual threat to survival. Thus, costs of mounting a large stress response may outweigh benefits (Partecke et al. 2006; Bonier et al. 2007; Atwell et al. 2012). However, a larger stress response could also confer fitness benefits in an urban environment, for example, if an altered predation regime favors a robust survival response (Boonstra 1998).

Comparisons between GC levels in rural and urban populations yield some support for the idea that HPA down-regulation may promote persistence in the face of anthropogenic activity (Partecke et al. 2006; Atwell et al. 2012), while also suggesting that human disturbance may overwhelm behavioral and physiological coping mechanisms and induce chronic stress (Walker et al. 2005; Bonier et al. 2007; Wasser et al. 2007; Hayward et al. 2011). However, studies on the relationship between urbanization and GC concentrations have yielded no consistent patterns (Fokidis et al. 2009; Bonier 2012 for review), and the majority of studies have actually reported null relationships between urbanization level and variation in stress physiology (Fokidis et al. 2009; Chavez-Zichinelli et al. 2010; Bókony et al. 2012; Bonier 2012). Conflicting results may be explained by the specific ecology and evolutionary history of species used in analyses, which may influence sensitivity and responses to anthropogenic disturbance (Bonier et al. 2007; Fokidis et al. 2009).

We explored how urban disturbance affects stress physiology and body condition metrics within an urban population of song sparrows (*Melospiza melodia*). Despite extensive and pioneering work on the effects of urbanization on stress physiology in birds, including work by Veronika Bókony, Frances Bonier, Bob Fokidis and Steve Schoech, most studies to date involve broad-scale comparisons between one urban and rural or suburban population (but see Bonier 2012 for review). Heterogeneity in disturbance regime exists within urban landscapes due to presence of both activity centers, adjacent to highways or shopping centers, and activity refuges, such as parks or reserves. Thus, as on the broader scale, individuals breeding within urban environments may display changes in stress physiology based on divergent selective pressures experienced. Our study had three objectives, which we expand below.

Firstly, we examined whether maximum corticosterone (CORT, the primary avian GC) concentrations after exposure to stress (acute CORT levels), baseline CORT concentrations, or the magnitude of the stress response (delta CORT; acute – baseline CORT) vary in male song sparrows (*Melospiza melodia*) as a function of urban habitat type (activity centers versus activity refuges). We were particularly interested in whether song sparrows displayed suppressed stress responses in activity centers, which could facilitate maintenance of reproductive activity despite frequent perturbation, and in whether or not baseline CORT would be elevated in disturbed areas. Acute CORT concentrations better reflect biological effects of elevation of hormone levels post-stressor, since receptor-binding and consequential phenotypic effects are proportional to absolute hormone concentration, irrespective of baseline CORT levels (Romero 2004). However, we retain delta CORT as a variable of interest because it reflects the responsiveness of the HPA axis to disturbance (the quantity of hormone released in excess of baseline levels), which may be suppressed given habituation to urban disturbance sources (Partecke et al. 2006). Delta CORT may be lower merely as a consequence of elevated baseline CORT, or because acute CORT levels are lower in the absence of changes in baseline physiology. Thus, we address both contingencies when interpreting our data.

Secondly, we evaluated whether stress physiology varies as a function of the noise environment. We were particularly interested in whether or not elevated noise would translate into elevation of stress hormone levels, which could be reflective of chronic stress. Noise may pose challenges for organism breeding in urban environments, and potentially induce chronic stress, by directly disturbing individuals, altering physiological processes (Barber et al. 2009; Kight and Swaddle 2011; Francis and Barber 2013), and interfering with communication (Brumm 2004; Brumm and Slabbekoorn 2005; Habib et al. 2007; Gross et al. 2010; Leonard and Horn 2012; Schroeder et al. 2012; Naguib et al. 2013). Indeed, past studies on the effect of noise on breeding birds have reported that noise is associated with reduced reproductive success, even in species common in urban areas (Halfwerk et al. 2011; Kight et al. 2012; Strasser and Heath 2013), and some studies have associated loud noise with elevated stress hormone levels (Hayward et al. 2011; Blickey et al. 2012; Strasser and Heath 2013). However, as for effects of urbanization in general, past studies report varying results regarding how loud noise effects stress physiology and body condition (Kight and Swaddle 2011 for review), with null results being common (Morgan et al. 2012; Payne et al. 2012; Proppe et al. 2013).

Finally, to more comprehensively assess whether song sparrows experience increased allostatic load as a function of anthropogenic disturbance, we examined whether birds displayed differences in a number of condition-related metrics as a function of urban habitat type and noise environment. We assessed variation in body mass as a means of examining whether birds breeding in distinct urban microhabitats differed in body reserves. In addition, we examined variation in feather quality as an indirect metric of condition at molt, since birds in better condition at molt may grow longer, more wear-resistant feathers (Harper 1999). Song sparrows in our study population are year-round residents, such that birds experience similar levels of anthropogenic disturbance year-round. Next, we tested for variation in hematocrit levels, since chronic stress associated with disturbance may result in anemia (reduced red blood cell count), thus interfering with oxygen carrying capacity (Davis et al. 2008; Bókony et al. 2012). Finally, we looked for evidence of differences in total antioxidant capacity (TAC), which may be indicative of greater oxidative challenge and heightened energetic demands in highly urbanized environments (Isaksson et al. 2005, 2007; Cohen et al. 2007; Møller et al. 2010).

METHODS

Study species and sites

We studied variation in the adrenocortical stress response in an urban population of song sparrows breeding in activity centers and refuges near the University of California, Riverside (UCR). Activity center sites were located on, and in the immediate vicinity of the UCR campus and the I-215/CA-60 freeway (center: 33°58'27.23"N 117°19'38.39"W). These birds are constantly exposed to disturbances including noise from air-conditioning units on buildings, passing traffic, and human conversation. Refuge sites included the 1,150 acre Box Springs Mountains Reserve (center: 33°57'56.71"N 117°17'7.86"W), located approximately 4 kilometers east of the UCR campus center, and the 1,550 acre Sycamore Canyon Wilderness Park (center: 33°56'14.11"N 117°18'59.11"W), located approximately 4 kilometers south of the UCR campus center. Although these reserves are entirely embedded within Riverside's urban matrix and show ample urban impacts on vegetation and various other attributes, birds captured within refuges are relatively buffered from anthropogenic disturbance sources. Vehicular traffic is prohibited, and recreational activity, including walking and biking, is allowed but entails light and sporadic human traffic.

We focused our study on territorial breeding males, which we captured in mist nets using conspecific playback. All males captured were actively breeding, as assessed by the presence of a cloacal protuberance. We captured birds from March 6 through April 18, 2011 and from February 26 through April 12, 2012, between 0700 h and 1500 h local time. We controlled for time and date effects in statistical analyses. Upon capture we banded each bird with an aluminum USGS band and with an unique color combination to allow for subsequent field identification.

The UCR Animal Care and Use Committee approved all animal use procedures (Protocol A-20100002E), and banding and blood sampling were additionally authorized by a USGS bird-banding sub-permit (23035-F) and a California state collecting permit (SC-11059).

Characterization of disturbance environment

To characterize the anthropogenic disturbance environment we unambiguously classified activity refuges and activity centers. Refuges were distinguished by park-land designation and were separated from, although embedded within, the surrounding urban matrix. Centers were located within the urban matrix, where heavy human activity occurs. In addition, we measured noise level in decibels (dB) on the territory of each male. We followed a protocol for measuring noise level that has been employed in studies examining effects of anthropogenic noise on bird song characteristics (Brumm 2004; Bermúdez-Cuamatzin 2009). We used a Digital Instruments SL-4023SD sound level meter (range, 30-130 dB; weight, A; fast response; IEC 61672 class 2) with a windshield ball. We recorded noise levels between 0600 h and 1300 h local time, corresponding to the time period in which sparrows were captured, with the majority of readings taken immediately after the sparrow was captured and processed. We took measurements for 5 minutes, with noise levels being logged every 5 seconds. For one minute each we pointed the sound level meter in each of the four cardinal directions, and straight upward. We flipped a coin to determine whether to start from north or south, and west was always followed by upwards. We took the average of all 5-second incremental measurements as a final metric of the noise environment. In addition, we extracted maximum noise, minimum noise, and the standard deviation of noise level across readings.

Stress response sampling protocol and measuring hematocrit

We measured the stress response using standard capture protocol (Wingfield et al. 1992; Bonier 2012), with an initial sample taken within 3 minutes of capture (representative of baseline CORT) and another sample taken after 30 minutes (representative of acute CORT). We confined birds in cloth holding bags in the interlude between sampling. We used 26-gauge needles and heparinized microcapillary tubes to withdraw small blood samples (~80 μl) from the brachial vein. We stored blood samples on ice in the field. Plasma was separated from cell fraction via centrifugation for 12 minutes at 11,000 rpm. We determined hematocrit levels immediately following centrifugation by measuring the percentage of the total blood column occupied by erythrocytes. We stored plasma at -30°C until performing CORT radioimmunoassays.

Measuring body mass and feather quality

At the time of capture, we measured body mass (± 0.1 g) using a digital scale, unflattened wingchord (± 1 mm) using a wing scale, and tarsus length (± 0.01 mm) using digital calipers. We used mass alone as a metric of body reserves, instead of using least-square residuals or a more sophisticated metric, such as the scaled mass index (Peig and Green 2009, 2010; Bókony et al.

2012), because tarsus length and body mass were not strongly correlated in our dataset (Spearman correlation: $r_s = 0.08$, $N = 86$, $P = 0.44$).

In addition, we scored feather wear on a 0 to 4, where 0 corresponds to no wear, 1 to a trace of wear, 3 to moderate wear, and 4 to heavy wear (Ralph et al. 1993). Feather wear indicates the degree to which primary feathers (main flight feathers) are abraded away, and thus is reflective of primary feather quality. Birds in better condition at molt may grow both longer and more wear-resistant feathers (Harper 1999; Dawson et al. 2000). Thus, we conducted a principal components analysis on wingchord and feather wear, which were negatively correlated in our dataset (Spearman correlation: $r_s = -0.38$, $N = 89$, $P < 0.001$), to combine these variables into a single metric indicative of condition at molt. This metric, PC1, was positively loaded on wingchord and negatively loaded on feather wear (Table 6.1). Thus, we interpret higher PC1 scores as reflecting higher quality, more wear-resistant feathers and superior condition at molt.

Corticosterone radioimmunoassays

We conducted corticosterone assays using a MP Biomedical I^{125} radioimmunoassay (RIA) kit (07-120103), which has been previously validated for use in *M. melodia* (Newman et al. 2010). We followed kit instructions with the exception that baseline plasma samples were diluted 1:100 with steroid diluent (5 μl plasma: 495 μl diluent), and stressed samples 1:200 (5 μl plasma: 995 μl diluent). We assayed all samples in duplicate, and included high and low controls provided by the kit manufacturer in each assay. We randomly distributed samples from refuge and center males between assays. Intra-assay coefficients of variation averaged 11.7% and inter-assay coefficients of variation were 8.1 % and 11.0% for high and low controls, respectively.

Antioxidant assay

For a subset of males captured in 2012 (N = 52, 18 in activity refuges and 36 in activity centers), we performed an antioxidant assay to determine total antioxidant capacity (TAC) of plasma samples taken within 3 minutes post capture. Specifically, we used the OXY-adsorbent assay kit commercially available through Diacron International (Costantini et al. 2007), and a Spectra Max Plus 96-well plate reader capable of temperature regulation. Preparation for the assay was done on ice to avoid oxidation of samples. We diluted plasma 1:100 with distilled water (2 μL plasma: 198 μL water). We then generated a standard curve consisting of solutions capable of neutralizing 0, 115, 230, and 460 mM of hypochloric acid (HOCl), a generic antioxidant. We added 200 μL of HOCl and 5 μL of diluted plasma (or standard) to the wells of a microplate, and performed a pre-read of the plate to control for minimal variation in sample absorbance. We incubated the plate for 5 minutes at 37°C, and then added 2 μL of a chromogenic solution, mixed thoroughly and immediately read absorbance at 505 nm. We report results in terms of mM of HOCl neutralized. We randomly distributed samples from activity refuge and activity center sparrows across the 96-well plate. All samples were assayed in duplicate in a single assay. Intra-assay coefficients of variation averaged 7.15%.

Statistical analysis

We conducted all statistical analyses in R 2.15.2 (R Core Team, 2012). Specifically, we used linear mixed effects models (LMM) in the lme4 package, which allowed us to incorporate bird identity as a random effect (five individuals were captured across both years of the study). Two-tailed tests were applied for all analyses. We report beta estimates (± SE), and additionally include effect size estimates (Cohen's d) and associated 95% confidence intervals for variables retained in final models predicting CORT levels and other condition metrics from urban habitat type (Nakagawa and Cuthill 2007; Table 6.1).

First, we used separate model to determine whether the magnitude of the stress response (delta CORT), acute CORT, or baseline CORT concentrations were significantly different in activity centers than in activity refuges. We included time and date of capture as fixed effect covariates in these, and all subsequent, models. We log transformed delta, acute, and baseline CORT to normalize the distribution of residuals. We used a Satterthwaite approximation (implemented by the lmerTest package of R) to estimate degrees of freedom for LMM models

In addition, the study design was unbalanced due to higher sampling rates of males in activity centers, especially early in the season. Specifically, we captured 85 males (80 unique males) across two years, with 5 individuals captured in both 2011 and 2012. Due to greater ease of capturing males in activity centers, we captured 55 males in activity centers and 25 in activity refuges. Variances in the magnitude of the stress response (delta CORT), acute CORT, baseline CORT, body mass, hematocrit, feather PC1 and antioxidant capacity did not differ between habitat types (Levene's test: $F_{1,80} = 1.79$, $P = 0.18$; $F_{1,81} = 0.14$, $P = 0.71$; $F_{1,85} = 2.28$, $P = 0.13$; $F_{1,83} < 0.001$, $P = 0.98$; $F_{1,86} = 0.84$, $P = 0.36$; $F_{1,86} = 0.33$, $P = 0.57$, $F_{1,52} = 0.07$, $P = 0.80$; respectively). Thus, unequal sample sizes should not cause a problem for statistical analyses. Nevertheless, we caught males in activity centers an average of 8.07 days earlier in the season (LMM: $F_{1,86} = 7.03$, $\beta = -8.07 \pm 3.04$ (SE), $P = 0.01$) and 0.71 hours earlier in the day (LMM: $F_{1,86} = 4.21$, $\beta = -0.71 \pm 0.35$, $P = 0.04$) than males in refuges, which could confound results. We resolved this problem by producing a subset of data in which to repeat analyses.

In the subset data, we retained all refuge males except for the second observation on one individual that was caught both in 2011 and 2012, and eliminated males caught earlier in the season in activity centers, such that the sampling period extended from March 5 to April 17, instead of beginning in late February. We then paired birds caught in centers and refuges according to capture time and date (N = 25 in refuges and N = 25 in centers). In this dataset, date and time of capture did not differ between habitat types (Linear model (LM): $F_{1,48} = 0.02$, $R^2 = 0.0004$, $\beta = -0.40 \pm 2.92$, $P = 0.89$; $F_{1,48} = 1.06$, $R^2 = 0.02$, $\beta = -0.44 \pm 0.43$, $P = 0.31$, respectively). We employed linear models to rerun analyses in the subset data, since this dataset did not contain duplicated individuals.

Further, we used linear mixed effects (entire dataset) and linear (subset data) models to assess whether body mass, hematocrit levels, total plasma antioxidant capacity, or feather quality differed between habitat types or with baseline or delta CORT levels. We did not use acute CORT in this analysis because delta and acute CORT were highly correlated in our dataset (Spearman correlation: $r_s = 0.87$, $N = 82$, $P < 0.001$), which could create problems with collinearity. Acute CORT was also correlated with baseline CORT (Spearman correlation: $r_s = 0.50$, $N = 82$, $P < 0.001$), whereas delta CORT was not (Spearman correlation: $r_s = 0.11$, $N = 82$, $P = 0.28$). For modeling body mass, hematocrit levels, and feather quality we entered habitat type and baseline and delta CORT as fixed effects. We also included interactions between habitat type and CORT variables, since we were interested in whether CORT levels correlate differently with body condition in the two habitat types. For modeling antioxidant capacity, birds were not duplicated in the dataset, so we used a linear model with habitat type and CORT variables as fixed effect predictor variables. We log transformed antioxidant capacity, and again included interactions between habitat type and CORT variables. Additionally, we applied Spearman correlations to determine whether body mass, antioxidant capacity, hematocrit, and feather quality were correlated in our dataset.

Finally, we assessed whether variation in disturbance level as reflected by noise measurements predicted delta CORT, acute CORT, baseline CORT, body mass, hematocrit levels, feather quality, or total antioxidant capacity, either across habitat types or within activity centers alone. To avoid collinearity in correlated sound level variables (average noise, maximum noise, minimum noise, and standard deviation) we first reduced these variables to two orthogonal explanatory variables using principal component analysis (Table 6.1).

Table 6.1. Principal components analysis on feather quality and noise level variables. Entries for each variable are factor loadings.

	PC1	PC2
Feather quality PCA		
Feather wear	-0.70	
Wingchord	0.70	
Eigenvalue	1.18	
Cumulative proportion of variance	0.69	
Noise level PCA		
Mean noise	0.61	0.02
Maximum noise	0.43	0.61
Minimum noise	0.61	-0.10
Standard deviation of noise level	-0.27	0.79
Eigenvalue	2.62	1.25
Cumulative proportion of variance	0.66	0.97

The first PC axis (PC1) had high positive loadings for average noise, maximum noise, and minimum noise level, and thus can be interpreted as relating to the magnitude of noise level (Table 6.1). The second PC axis (PC2) had high positive loadings for the standard deviation of noise level and maximum noise, and thus can be interpreted as reflecting variation in noise level (Table 6.1). We then applied linear mixed effects (entire dataset) and linear models (subset data) with CORT variables, other condition metrics, and total antioxidant capacity as the dependent variable in separate models. We entered the two sound level PCs as fixed effects, and initially included a quadratic term for noise PCs in models predicting CORT concentrations to test for a non-linear relationship with noise environment.

RESULTS

The stress response with respect to habitat type

Stress-induced (acute) CORT concentrations were significantly higher than baseline CORT concentrations (t-test: $t_{1,\,177} = -14.747$, $P < 0.001$) in both urban habitat types. Acute, baseline and delta CORT levels did not significantly differ between urban habitat types in either the entire or subset datasets (Table 6.2). However, activity center males tended to have lower acute than activity refuge males in the subset dataset, and lower delta CORT than activity refuge males in both datasets (Table 6.2). Date of capture had a marginally significant effect on acute CORT within the entire dataset (Table 6.2), but had no effect on acute CORT levels in the subset data (LM: $P > 0.10$; Appendix 6, Table 6.A1). Time of capture had no effect on acute CORT levels, and date and time of capture had no effect on the magnitude of the stress response in either dataset (LMM, LM: $P > 0.10$; Appendix 6, Table 6.A1). In the entire dataset, the best model for predicting baseline CORT included time of capture alone, with higher baseline CORT concentrations earlier in the day (Table 6.2), but baseline CORT concentrations also tended to be higher earlier in the season (Table 6.2). In the subset data, sampling date had no effect on baseline CORT (LM: $F_{1,}$ $P > 0.1$; Appendix 6, Table 6.A1), perhaps due to the narrower window of sampling dates encompassed. However, baseline CORT still declined later in the day (Table 6.2). We report the mean ± SE of CORT levels in activity centers and refuges in Table 6.3.

Other condition-related variables with respect to habitat type

In both the entire and subset datasets, body mass, hematocrit and total antioxidant capacity did not differ as a function of habitat type (LMM, LM: $P > 0.10$; Table 6.2), and the only significant effect of time and date of capture was a negative effect of time on hematocrit levels within the subset data (Table 6.1; Table 6.A2). However, in the entire dataset, sparrows breeding in activity centers had higher feather PC1 scores, although this relationship was not significant in the subset data (Table 6.2). There was no indication that CORT levels interaction with habitat type to predict any of our other condition metrics (LMM, LM: $P > 0.10$; Appendix 6, Tables 6.A2 and 6.A3). However, although total antioxidant capacity was not correlated with delta CORT (LM: $P > 0.10$; Appendix 6, Table 6.A2), it was positively predicted by baseline CORT concentrations (LM: $F_{1,52} = 4.89$, $R^2 = 0.09$, $\beta = 0.002 \pm 0.001$, $P = 0.03$; Figure 6.1). No other condition metric was related to CORT levels (LMM/LM: $P > 0.10$; Appendix 6, Tables 6.A2 and 6.A3), and condition-related metrics were uncorrelated in our dataset (Spearman rank correlation: $r_s = 0.10$, $N = 52$, $P = 0.49$; Table 6.4). We report mean ± SE levels of condition related variables in Table 6.3.

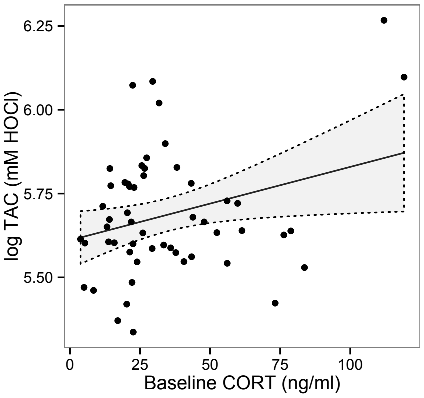

Figure 6.1. Linear regression of the relationship between baseline CORT and total antioxidant capacity (TAC). The shaded region shows the 95% confidence interval.

Plasma CORT, and other condition metrics with respect to environmental noise

Activity refuge territories had significantly smaller PC1 scores than activity center territories (t-test: $t_{1,87} = -18.52$, $P < 0.001$; Figure 6.2a), reflecting the quieter noise environment found in refuges, whereas PC2 did not differ with habitat type (t-test: $t_{1,65} = -0.02$, $P = 0.98$; Figure 6.2b). We report mean ± SE levels of noise PCs for activity centers and refuges in Table 6.3.

Table 6.2. Final LMM and LM models predicting plasma CORT levels, body mass, hematocrit, feather PC1 and total antioxidant capacity (TAC) with respect to habitat type in the entire and subset datasets

	N	Intercept ± SE	β ± SE	F	Denom (df)	P	Cohen's d (95% CI)
Entire dataset							
Acute CORT[a]	83, 78[b]	5.06 ± 0.14					
Habitat type			-0.18 ± 0.11[c]	2.45	74.99	0.12	-0.37 (-0.81; 0.07)
Date			-0.01 ± 0.004	3.01	42.48	0.09	-0.41 (0.03; -0.85)
Baseline CORT (ng/ml)	87, 82	5.15 ± 0.55					
Habitat type			-0.03 ± 0.16[a]	0.03	80.36	0.85	-0.01 (-0.43; 0.42)
Time			-0.14 ± 0.05	8.74	80.14	0.004	-0.76 (-1.20; -0.32)
Date			-0.01 ± 0.005	3.33	74.88	0.07	-0.47 (-0.90; -0.04)
Delta CORT (ng/ml)	82, 77	4.60 ± 0.11					
Habitat type			-0.23 ± 0.13	3.28	79.99	0.07	-0.44 (-0.88; 0.004)
Body mass (g)	85, 80	20.10 ± 0.18					
Habitat type			0.17 ± 0.22	0.60	77.94	0.44	0.07 (-0.36; 0.50)
Hematocrit (%)	88, 83	48.40 ± 0.76					
Habitat type			0.88 ± 0.91	0.95	86.00	0.33	0.23 (-0.19; 0.65)
Feather PC1	88, 83	-0.42 ± 0.23					
Habitat type			0.59 ± 0.27	4.77	80.77	0.03	0.55 (0.12; 0.98)
TAC (mM HOCl)	54	5.71 ± 0.04					
Habitat type			-0.03 ± 0.05	0.34	52	0.56	-0.17 (-0.71; 0.37)
Subset data							
Acute CORT (ng/ml)	50	4.87 ± 0.10					
Habitat type			-0.26 ± 0.14	3.28	48	0.076	-0.51 (-1.08; 0.07)
Baseline CORT (ng/ml)	50	4.75 ± 0.73					
Habitat type			-0.12 ± 0.19	0.11	47	0.74	-0.17 (-0.74; 0.40)
Time			-0.13 ± 0.06	4.46	47	0.04	-0.60 (-1.17; -0.001)
Delta CORT (ng/ml)	50	4.60 ± 0.12					
Habitat type			-0.33 ± 0.16	4.04	48	0.05	-0.57 (-1.13; 0.02)
Body mass (g)	49	20.12 ± 0.12					
Habitat type			0.34 ± 0.25	1.85	47	0.18	0.39 (-0.20; 0.95)
Hematocrit (%)	50	57.41 ± 4.46					
Habitat type			1.43 ± 1.15	2.39	46	0.13	0.35 (-0.23; 0.91)
Time			-0.74 ± 0.38	3.89	46	0.05	-0.56 (-1.12, 0.03)
Feather PC1	50	-0.51 ± 0.22					
Habitat type			0.22 ± 0.31	0.50	48	0.48	0.20 (-0.37; 0.76)

[a] Activity center relative to activity refuge habitat.

Table 6.3. Means and standard error of corticosterone concentrations, other condition indices and noise PCs in activity centers and refuges in both the entire and subset datasets

	Center (Mean ± SE)	Refuge (Mean ± SE)	P (>F)
Entire dataset			
Acute CORT (ng/ml)	127.70 ± 7.42	144.93 ± 60.68	0.12
Baseline CORT (ng/ml)	38.91 ± 3.49	31.66 ± 3.19	0.85
Delta CORT (ng/ml)	89.64 ± 6.22	114.13 ± 12.39	0.07
Body mass (g)	-0.0002 ± 0.11	-0.15 ± 0.16	0.27
Hematocrit (%)	49.28 ± 0.48	48.40 ± 0.83	0.33
Feather PC1	0.19 ± 0.15	-0.40 ± 0.22	0.03
Antioxidant capacity (mM HOCl)	300.45 ± 11.05	299.66 ± 12.21	0.44
Noise PC1 (dB)	1.00 ± 0.12	-1.93 ± 0.14	<0.001
Noise PC2 (dB)	-0.04 ± 0.15	0.002 ± 0.22	0.98
Subset data			
Acute CORT (ng/ml)	116.49 ± 13.02	143.27 ± 12.22	0.076
Baseline CORT (ng/ml)	31.03 ± 5.04	31.66 ± 3.19	0.67
Delta CORT (ng/ml)	85.46 ± 11.25	113.94 ± 12.32	0.05
Body mass (g)	20.46 ± 0.19	20.12 ± 0.16	0.18
Hematocrit (%)	50.52 ± 0.88	48.76 ± 0.78	0.13
Feather PC1	-0.29 ± 0.22	-0.51 ± 0.22	0.48
Noise PC1 (dB)	1.21 ± 0.18	-1.93 ± 0.14	<0.001
Noise PC2 (dB)	-0.33 ± 0.21	-0.12 ± 0.21	0.47

Table 6.4. Spearman correlations between condition metrics

	Hematocrit	Feather PC1	Total antioxidant capacity
Body mass	$r_s = 0.06$, N = 85, P = 0.57	$r_s = 0.12$, N = 85, P = 0.26	$r_s = 0.11$, N = 52, P = 0.42
Hematocrit	---	$r_s = -0.03$, N = 88, P = 0.77	$r_s = 0.02$, N = 54, P = 0.87
Feather PC1	---	---	$r_s = -0.07$, N = 54, P = 0.60

Despite differing among habitat types, magnitude of noise level (noise PC1) did a poor job of predicting acute and delta CORT across activity center and refuge males in both datasets, and also was unrelated to acute or delta CORT within activity center males alone (LM, LMM: $P > 0.10$ in all cases; Appendix 6, Table 6.A4 and 6.A5). As for acute and delta CORT, magnitude of noise (noise PC1) did not predict baseline CORT concentrations across activity center and refuge males in either dataset (LM, LMM: $P > 0.10$ in all cases; Appendix 6, Table 6.A6). However, within activity center males alone, males tended to have lower baseline CORT levels on noisier territories (LMM: $F_{1, 57} = 3.76$, $\beta = -0.18 \pm 0.09$, $P = 0.058$; Figure 6.3), when controlling for

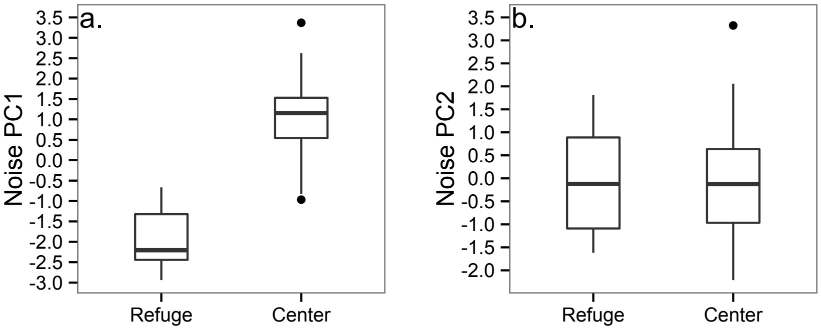

Figure 6.2. Boxplots showing the distribution of noise levels between urban habitat types. Noise PC1 reflects the magnitude of noise levels, whereas noise PC2 reflects the standard deviation of noise levels. Whiskers extend from the first and third quartiles to the highest value within 1.5 × IQR (Interquartile range).

significant effects of time and date of capture (LMM: $F_{1,57} = 7.71$, $\beta = -0.18 \pm 0.06$, $P = 0.007$; $F_{1,57} = 4.44$, $\beta = -0.01 \pm 0.006$, $P = 0.04$, respectively). Variation in noise level (noise PC2) failed to predict acute, baseline or delta CORT concentrations across habitat types or within activity centers (LM, LMM: $P > 0.10$ in all cases; Appendix 6, Table 6.A4, 6.A5, and 6.A6).

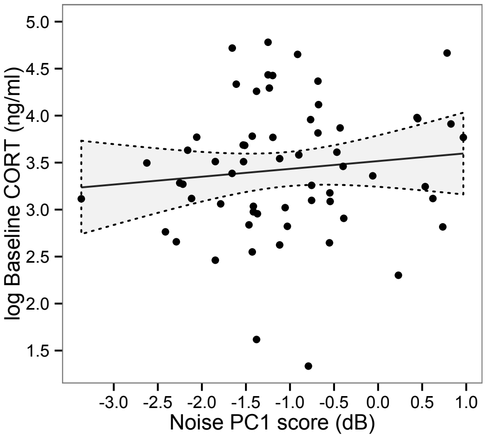

Figure 6.3. Relationship between noise PC1 (magnitude of noise) and baseline CORT concentrations within activity centers. The shaded region shows the 95% confidence interval.

The only significant effect of noise environment on body mass was a negative effect of variation in noise level (noise PC2) within the subset data, independent of capture time and date

(LM: $F_{1, 47} = 4.70$, $R^2 = 0.09$, $\beta = -0.25 \pm 0.11$, $P = 0.03$; Appendix 6, Table 6.A7). Similarly, the only relationship between noise environment and hematocrit was within the subset data, but this correlation was positive (LM: $F_{1, 47} = 4.87$, $R^2 = 0.09$, $\beta = 1.20 \pm 0.54$, $P = 0.03$), indicating that males breeding on territories with more variable noise levels had higher hematocrit. Within the entire dataset, noise environment did not affect body mass or hematocrit, either across habitat types or within activity center males alone (LMM: $P > 0.10$; Appendix 6, Table 6.A7 and 6.A8). Rather, body mass increased with time of capture within activity center males alone (LMM: $F_{1, 41} = 7.27$, $\beta = 0.20 \pm 0.07$, $P = 0.01$; Appendix 6, Table 6.A7). Feather PC1 and total antioxidant capacity were not predicted by noise environment either across habitat types or within activity center males alone (LM: $P > 0.05$, Appendix 6, Tables 6.A9 and 6.A10).

DISCUSSION

In accordance with the majority of work done thus far with respect to effects of urbanization on CORT concentrations in birds (Bókony et al. 2012a; Bonier 2012 for review), we found only weak evidence that the adrenocortical stress response varies with discrete differences in disturbance level, or the noise environment. Lack of variation between urbanization level and CORT concentrations in song sparrows may reflect the fact that this species is an urban adapter species, which has adjusted to unique selective pressures experienced in the urban realm and which does not perceive anthropogenic disturbance as stressful.

However, *M. melodia* males in activity centers did tend to have smaller stress responses (delta CORT) relative to males in activity refuges ($P = 0.05$ and $P = 0.07$ in the subset and entire dataset, respectively). The marginally significant difference between delta CORT in the two urban habitats was driven by a tendency towards lower acute CORT ($P = 0.076$ and $P = 0.12$, in the subset and entire datasets, respectively), rather than elevated baseline CORT (mean nearly equivalent between habitats). Some previous studies have reported negative correlations between the magnitude of the stress response and urbanization, for instance in European blackbirds (Partecke et al. 2006), and dark-eyed juncos (*Junco hyemalis*) (Atwell et al. 2012), and have suggested that suppression of the HPA axis may aid in adaptation to high-disturbance environments. However, other studies have found contrasting results (Fokidis et al. 2009; Bókony et al. 2012; Bonier 2012), and due to the marginally significant nature of our result it must be viewed with caution.

Further, rather than being an adaptive response to anthropogenic disturbance, the trend towards lower delta CORT in activity centers could be explained by differences in breeding phenology or brood value between males breeding in the two urban habitat types. Indeed, a recent review indicates that breeding phenology and brood size often differ between birds breeding in environments with different levels of urbanization (Chamberlain et al. 2009). In many species, including song sparrows, acute elevation of CORT is suppressed during the nestling stage (Wingfield 1984; Wingfield et al. 1995; M. Grunst, unpublished data). Moreover, past research on both the inter-specific and intra-specific levels has demonstrated that increased brood value is related to greater suppression of the stress response during the breeding season (Lendvai et al. 2007; Bókony et al. 2009). Thus, potential breeding-related explanations for the trend towards lower delta CORT in activity centers include advancement of breeding in activity centers such that more individuals were in the nestling stage when captured, a more favorable breeding environment in activity centers that allowed for larger brood size, or higher nest predation rates in activity refuges that precluded many pairs in refuges from reaching the nestling stage. Unfortunately, we lack precise information about breeding stage, brood size, or nest predation rates. Importantly, if advancement of breeding or higher brood values does explain the trend towards lower delta CORT in activity centers, these explanations still suggest that song sparrows have adapted to the urban environment, and may actually perceive activity center habitat as more favorable than refuge habitat, despite higher disturbance levels.

In contrast to the magnitude of the stress response, sparrows breeding in different urban habitats showed no evidence of differences in baseline CORT concentrations, suggesting that anthropogenic disturbance does not result in chronic stress or increased allostatic challenge in activity center males. This result contrasts with positive correlations reported between baseline CORT and urbanization in tree sparrows (*Passer montanus*) (Zhang et al. 2011) and male white-crowned sparrows (*Zonotrichia leucophrys*) (Bonier et al. 2007), and with studies that link higher baseline CORT to tourism and logging activity (Wasser et al. 1997; Walker et al. 2005). However, past studies also report null relationships between baseline CORT and urbanization (Partecke et al. 2006; Fokidis et al. 2009; Atwell et al. 2012; Bókony et al. 2012; Bonier 2012). Discrepancy in covariation between baseline CORT and anthropogenic disturbance may relate to interspecific differences in the ability to adjust physiology and habituate to disturbance or to differences in the allostatic challenges that urbanization poses for different species (Bonier 2012). Energetic demands associated with foraging may increase in urban areas due to decreased foraging substrate and increased coverage by cement and other impervious surfaces (Zhang et al. 2011; Bonier 2012). However, Riverside is surrounded by xeric coastal sage scrub, such that urbanization may also enhance habitat quality and ease foraging challenges (Fokidis et al. 2009).

As for baseline CORT, body mass, hematocrit, and antioxidant capacity did not differ between habitat types, again suggesting that sparrows in activity centers are not experiencing chronic stress. Similarity in body mass suggests maintenance of comparable energetic reserves, whereas similar hematocrit levels indicate that birds are not becoming anemic due to chronic stress imposed by disturbance (Fair et al. 2007; Davis et al. 2008). Similarity in antioxidant capacity is consistent with the interpretation that activity center sparrows do not face increased oxidative challenge, which could arise in response to urban pollution, increased foraging effort in degraded habitat, or stress related to human activity (Isaksson et al. 2005, 2007; Møller et al. 2010). In contrast to our results, great tits (*Parus major*) do display up-regulated antioxidant capacity in urban areas (Isaksson et al. 2007). Rather than greater oxidative challenges, high antioxidant capacity can indicate better condition and a greater capacity to combat oxidative challenge (Costantini and Verhulst 2009; Monaghan et al. 2009). Thus, to fully interpret the meaning of elevated antioxidant capacity one needs to directly measure oxidative damage (Costantini and Verhulst 2009). Unfortunately, we were unable to assess oxidative damage. Thus, urban sparrows could face higher oxidative challenge, but be unable to increase antioxidant capacity due to poor body condition. However, contrary to this interpretation, other condition metrics did not differ between habitat types. Indeed, the relatively close proximity of activity centers and refuges, and high levels of regional air pollution, may mean that sparrows breeding in both urban habitat types face substantial oxidative challenges.

Despite similarity in metrics of current condition, within the entire dataset we did find that birds in activity centers had higher feather quality than refuge birds. This result suggests that birds in activity centers were in better condition at molt (Harper 1999; Dawson et al. 2000), and that activity center habitat may be of higher quality than refuge habitat, despite higher anthropogenic disturbance levels. On the other hand, the relationship between feather quality and habitat type was not significant in the subset data in which date of capture was controlled. Therefore, although we detected no effect of capture date on feather quality, it is possible that correlations between date of capture, habitat type, and some other variable that affects feather quality is influencing results within the entire dataset.

Noise environment was unrelated to acute or delta CORT levels, suggesting that, if differences in delta CORT between urban habitat types do reflect meaningful variation, noise cannot be the selective agent responsible for the differences. Rather than noise, other disturbance-related attributes of refuge and center habitat, such as differences in human foot traffic, may explain the trend towards decreased delta CORT in activity center birds. However, we also recognize that our ability to detect an effect of noise on stress physiology may have been limited by the fact that our noise measurements represent “snap shots” of the noise environment.

Nevertheless, we did find that activity centers males breeding on noisier territories tended to have lower baseline CORT. This result contrasts to some past studies that link loud noise to elevated baseline CORT (Anderson et. al. 2011; Hayward et al. 2011; Kight and Swaddle 2011; Blickley et al. 2012; Strasser and Heath 2013). However, other studies have reported null or even negative correlations between the noise environment and baseline CORT (Kight and Swaddle 2011; Crino et al. 2013; Strasser and Heath 2013). Although elevated baseline CORT has most frequently been linked to chronic stress and negative fitness ramifications, lower baseline CORT has also been associated with chronic stress (Rich and Romero 2005; Cyr and Romero 2007). Thus, the association between lower baseline CORT and loud noise within activity centers could arise due to chronic stress experienced at very high disturbance levels. However, despite correlating with baseline CORT, magnitude of noise (noise PC1) was unrelated to body mass, hematocrit, feather quality and antioxidant capacity, suggesting that the negative association between loud noise and baseline CORT may not reflect pathology. As an alternative to chronic stress, lower baseline CORT in males breeding on loud territories may reflect suppression of the HPA axis to help ameliorate negative repercussions of perturbation by loud noise, as also suggested for the tendency towards lower magnitude stress responses in activity center males.

However, without manipulative experiments we cannot dissociate direct effects of noise on the HPA axis from other causative agents (Summers et al. 2011; Blickley et al. 2012).

For instance, baseline CORT may support energetically demanding breeding activity by regulating glucose metabolism (Sapolsky et al. 2000; Landys et al. 2006). Thus, another possibility is that males breeding on loud territories are investing less into breeding. Indeed, loud territories may be perceived as poor quality habitat, and be occupied by lower quality or less experienced individuals (Bayne et al. 2008; Habib et al. 2007; Slabbekoorn and Ripmeester 2008; Halfwerk et al. 2011).

Unlike noise magnitude (noise PC1), variation in noise (noise PC2) did not differ between habitat types, and thus might not be expected to explain habitat-associated differences in the HPA axis. Indeed, variation in noise was unrelated to differences in stress physiology. Nevertheless, variation in noise was negatively related to body mass in the subset data. Thus, variation in noise may be associated with lower body mass independent of changes in stress physiology, and multiple measures of physiological stress may be decoupled. Indeed, none of our condition-related metrics were significantly correlated, corroborating this conclusion. The negative association between noise PC2 and body mass could arise because unpredictable noise events are more likely to startle an organism and generate a flight-or-fight response (analogous to a response to predation risk) than predictable noise (Francis and Barber 2013). Thus, individuals subject to unpredictable noise may spend less time foraging and more time engaged in vigilance (Gavin and Komers 2006; Quinn et al. 2006; Francis and Barber 2013), or may maintain lighter body mass as a means of facilitating escape behavior (Brodin 2007; Cresswell 2008). In addition, noise PC2 was positively related to hematocrit within the subset data. Rather than suggesting that a variable noise environment can induce anemia, this result suggests that oxygen-carrying capacity may be up-regulated in variable noise environments. Thus, increased hematocrit could reflect higher activity levels, perhaps induced by a more dynamic noise environment (Fair et al. 2007).

In conclusion, our research contributes to a growing suite of studies that examine variation in the adrenocortical stress response and other physiological condition metrics with respect to urbanization. We detected only weak evidence of differences in stress physiology or condition metrics with respect to discrete urban habitat types or noise environment. Nevertheless, there were trends towards both smaller stress responses (delta CORT) in activity center males, and lower baseline CORT in activity center males breeding on loud territories. Although other explanations are possible, these findings tend to support the hypothesis that suppression of the stress response in urban areas may facilitate maintenance of health and reproductive activity. Further, lack of association between body mass, total antioxidant capacity, and hematocrit levels and either urban habitat type or magnitude of noise level suggests that song sparrows are not pathologically affected by human disturbance. Direct fitness measurements would be needed to fully elucidate whether human disturbance affects habitat quality from the perspective of a song sparrow. However, overall, results suggest that song sparrows are an urban adapter species that have adjusted to unique selective pressures encountered in the urban realm.

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GENERAL CONCLUSION

In this dissertation, I integrated behavioral studies with field endocrinology to grant insight into intraspecific variation in parental effort and sexual signaling. More specifically, I explored alternative hypotheses regarding how variation in maternal and paternal behavior is related to variation in the sexually selected trait of song complexity. In addition, I examined whether variation in the adrenocortical stress response correlates with song complexity, parental behavior, and anthropogenic disturbance levels, which could suggest a proximate control mechanism underlying sexual signaling dynamics, differential parental effort and adaptation to urban environments. Finally, and essentially, I explored the potential for predation risk to induce correlations between song complexity, the stress response, and parental behavior. I proceed to summarize the important conclusions arising from each of my dissertation chapters.

First, in Chapter 1, I linked variation in the adrenocortical stress response to song complexity and other male quality and fitness metrics. Whereas most previous studies have reported patterns of negative covariation between CORT levels and expression of sexually selected traits, I found that two dimensions of song complexity in *M. melodia*, song repertoire size and song syllable diversity, correlated oppositely with the magnitude of the stress response. Although the correlations observed may also have arisen during adulthood, this result suggests that only certain attributes of complex sexually selected traits may be subject to the actions of developmental stress, communicate developmental stress resistance, and negatively correlate with the magnitude of the stress response. Other components of sexually selected traits may communicate alternative information regarding male phenotypic or genetic quality (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994; Johnstone 1996; Candolin 2003; Jawor and Breitwisch 2004). Further, large stress responses may confer fitness benefits. Thus, one might sometimes expect high quality individuals to express large stress responses. Song repertoire size and song syllable diversity also correlated differently with other male quality and fitness metrics, corroborating the idea that the two song traits may convey distinct information regarding male phenotype. Consequently, females may assess multiple messages embedded in complex male songs when making decisions about mate choice, parental effort and risk-taking (Qvarnström et al. 2000; Candolin 2003; Rodriguez et al. 2013).

In Chapter 2, I demonstrated that male song complexity is associated with differences in paternal effort and thus communicates information about direct benefits to females (Greig-Smith 1982; Hill 1991; Palokangas 1994; Buchanan and Catchpole 2000). Moreover, females mated to males with varying song characteristics differentially responded to adult-directed predation risk near the nest, suggesting female use the information embedded in song to make decisions regarding parental risk-taking (Burley 1986, 1988; Harris and Uller 2009). However, rather than consistently supporting one hypothesis regarding the relationship between maternal and paternal effort and elaboration of sexual displays, song repertoire size and syllable diversity conveyed different information about male paternal quality and differentially predicted maternal risk-taking behavior. Specifically, song repertoire size positively predicted paternal performance and negatively predicted maternal risk-taking behavior, whereas syllable diversity negatively predicted paternal performance and positively predicted maternal risk-taking behavior. This result is consistent with results of Chapter 1 in supporting a multiple messaging function for the information content of song (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003). Finally, and perhaps most excitingly, I found that adult-directed predation risk induces a positive correlation between paternal nestling provisioning rates and song repertoire size, and magnifies differences in the provisioning rates of females mated to males with different song characteristics. Thus, the signaling content of song and dynamics of sexual selection may be altered in the context of predation pressure (Matessi et al. 2009).

In Chapter 3, I found only weak support for the hypothesis that suppression of the adrenocortical stress response mediates higher investment into parental activities (Romero 2002; Wingfield and Sapolsky 2003; Bókony et al. 2009). Specifically, the only negative correlation between the stress response and parental behavior was for female nestling stage nest attentiveness, and this result was not statistically significant. Further, males with larger, not smaller, magnitude stress responses displayed higher nestling stage nest attentiveness, and lesser reductions in nestling provisioning effort in response to adult-directed predation risk. Thus, whereas the result found in females is consistent with theory suggesting that smaller stress responses correlate with higher levels of parental effort, the result in males contradicts this theory. Significantly, I also found that the magnitude of the acute stress responses correlated positively with male repertoire size (Chapter 1), and that males with large song repertoires invested more paternally (Chapter 2). Taken together these results suggest that the sexually selected trait of song repertoire size advertises paternal performance, but that a large stress response, at least early in the season when most male song sparrows were captured, does not preclude high paternal performance relative to other males. Males with large song repertoires may resist negative effects of large stress responses on paternal performance by down-regulation of the stress response later in the nesting cycle, or via modulation of down-stream mechanisms (Breuner and Orchinik 2001; Breuner and Orchinik 2002a, 2002b; Breuner et al. 2003; Schmitd et al. 2012). Alternatively, males with large stress responses may be more prepared to evade predators, and thus may be able to sustain feeding rates under the threat of predation while facing lower survival costs. Finally, although results were contrary to predictions, I did find some support for the idea that predation pressure may induce correlations between the stress response and paternal effort, such that fitness consequences of differences in the stress response may be more pronounced under predation pressure.

In Chapter 4, I demonstrated that sex-specific differences in modulation of the HPA axis during the breeding season are consistent with the hypothesis that females, which alone incubation and have higher certainty of parentage, should down-regulate the stress response to a greater degree than males. Females displayed both lower baseline CORT concentrations and smaller magnitude stress responses than males (Wingfield 1984; O'Reilly and Wingfield 2005; Bókony et al. 2009). However, my results suggest that regulation of baseline CORT was not closely linked to mediation of parental activity in *M. melodia* since, despite being lower in females, baseline CORT concentrations did not vary within either sex as a function of changes in parental activities associated with nesting stage (Landys et al. 2006; Bókony et al. 2009). On the other hand, both sexes down-regulated the acute stress response during the nestling stage when brood value is elevated and parental demands intense, perhaps as a means of facilitating high parental effort.

In addition, in contrast to some past studies that report equalization of male and female CORT levels during the biparental nestling stage (Silverin et al. 1997; Holberton and Wingfield 2003), I found that sex differences in the stress response persisted in *M. melodia*. This result suggests that brood value remains higher for females than for males during the nestling stage, perhaps due to higher certainty of parentage or initial investment levels (costly ova) (Andersson 1994; Møller and Thornhill 1998; Westneat and Stewart 2003). Indeed, behavioral measurements indicated that females display higher parental effort and risk-taking than males during the nestling period, and this behavioral difference could potentially be mediated by differences in stress hormone levels that are maintained in the nestling stage. However, paternal contribution to nestling care did increase with brood size, suggesting that males may up-regulate paternal effort given a more valuable brood (Ardia 2007; Grundel 1987; Moreno et al. 1995).

In Chapter 5, I provide novel insight into the function of, and plasticity in, a unique female vocalization: the nest departure call (McDonald and Greenburg 1991). Results suggest that nest departure calls in song sparrows function to recruit males for nest-guarding duties during incubation off-bouts. Moreover, I found that females adaptively modulate production of nest departure calls based on male body condition, nest predator presence and nest height in a fashion consistent with balancing costs and benefits of calling. Plasticity in calling behavior likely exists because context-appropriate communication elevates fitness, whereas contextual mistakes in the decision to communicate result in fitness declines (Tuttle & Ryan 1981; Zuk et al. 1995; Zuk & Kolluru 1998). Thus, results offer insight into how communication systems used by biparental species may be plastically adjusted to maximize benefits and minimize costs of communicating, thereby increasing the probability of reproductive success.

Finally, in Chapter 6, I found only weak evidence that differences in the adrenocortical stress response exist within a heterogeneous environment, as a function of either discrete differences in anthropogenic disturbance levels or noise environment. This result is consistent with the majority of past work done on the relationship between stress physiology and urbanization in birds (Bonier 2012 for review), and may be explained by the fact that song sparrows are an urban adapter species that do not perceive human disturbance as overly stressful. However, I did find a trend towards lower acute stress responses in sparrows breeding in activity centers, with high levels of disturbance, relative to sparrows breeding in activity refuges, with low disturbance levels. Further, there was also a trend towards males breeding on very loud territories within activity centers having lower baseline CORT concentrations. Thus, although alternative explanations are possible, both smaller stress responses in activity center males and lower baseline CORT concentrations in males breeding on loud territories could serve to prevent pathological declines in health status and termination of reproductive activity in response to anthropogenic perturbation

(Partecke et al. 2006; Bonier et al. 2007; French et al. 2008; Atwell et al. 2012; Bonier 2012). Indeed, there was no evidence of pathology in activity center sparrows, as would be suggested by elevated baseline CORT and antioxidant levels, or reduced body mass and hematocrit, again supporting the conclusion that *M. melodia* successfully copes with anthropogenic stressors. This research contributes to a small, but growing, number of studies that examine the relationship between anthropogenic disturbance and adrenocortical stress physiology, and aids in motivating thinking about how changes in stress physiology may contribute to adaptation to the unique challenges encountered in urban environments (Bonier 2012).

In summary, this dissertation contributes to knowledge of sources of variation in parental effort and parental risk-taking, and sexual signaling dynamics. Central conclusions are as follows. First, only certain components of complex sexually selected displays, such as bird song, may correlate negatively with the magnitude of the stress response, perhaps due to differential actions of developmental stress. Second, only certain components of sexual displays may communicate direct benefits of paternal services. Moreover, given a multiple messaging function of sexual displays, females may differentially allocate (or compensate) with respect to only certain components of ornamentation. Thirdly, I found that the magnitude of the stress response, but not baseline CORT, correlated with certain metrics of parental effort and risk-taking, and thus may have the potential to mediate inter-sexual and inter-individual differences in parental effort. However, I found only weak evidence for the hypothesis that the stress response is negatively correlated with parental effort on the inter-individual level, with the stress response positively, rather than negatively, correlating with metrics paternal effort. Fourthly, predation risk may magnify or induce relationships between sexually selected traits (such as song complexity), the stress response, and parental effort, perhaps by elevating costs of parental care. Thus, both the strength of sexual selection, and fitness consequences of individual differences in adrenocortical stress physiology, may be magnified under predation pressure. Fifthly, nest departure calls of female song sparrows appear to function to facilitate male nest-guarding behavior, and females adjust calling behavior in fashions consistent with maximizing benefits and minimizing costs of communication. Finally, song sparrows are a successful urban adapter species and do not display evidence of pathology when breeding within an urban environment, and the adrenocortical stress response may aid in this adaptation.

The work presented in this dissertation suggests several directions for future research. Firstly, the link between stress experienced during development, expression of the stress response during adulthood, and the development of multifaceted sexually selected traits demands further investigation. Although some past work has shown that developmental stress can affect both the stress response and expression of sexual ornaments later in life (Spencer and MacDougall-Shackleton 2011), some sexually selected traits may be more vulnerable to early-life stress than others. With particular reference to the song sparrow (and other birds with complex song repertoires), future researchers could strive to elucidate the effects of manipulations of early-life stress on song repertoire size and syllable diversity. In addition, studies could further explore the signaling potential of song repertoire size and syllable diversity, and the role of these two traits in female choice. To this end, studies could examine the relationship between the song traits, extra-pair paternity, female choice, and male-male aggressive encounters. Creating playback of song with particular characteristics (e.g. constant song repertoire size, variable syllable diversity) would allow exploration of responses of females (solicitation or receptivity behavior) and males (aggressive behavior) to playback. In addition, more work is needed to clearly understanding the role of the adrenocortical stress response in mediating parental behavior and responses to anthropogenic disturbance. To further elucidate the role of the stress response in mediating parental behavior, it would be particularly powerful to manipulate CORT levels via silastic implants (or a similar technique), although achieving capture of birds closer to the timing of behavioral measurement would also be an improvement. In terms of the role of the stress response in aiding in urban adaptation, future work could investigate whether plastic or genetic changes in the hypothalamus-pituitary-adrenal axis underlie differences in the stress response observed, whether other traits coevolve with the stress response in urban environments, and, given the diversity of relationships between the stress response and urbanization observed (e.g. negative, positive, and null relationships reported), whether particular patterns of adrenocortical change are associated with particular species characteristics (e.g. phylogenetics, brain size, ecology). Finally, due to the paucity of studies on nest departure calls numerous directions of future research exist, including the effects of calling on context-dependent fitness, whether learning plays a role in call development, and if human disturbance interferes with the communicative function of calls.

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APPENDICES

APPENDIX 1

CHAPTER 1: Song repertoire size and song syllable diversity differentially predict the adrenocortical stress response in the song sparrow (*Melospiza melodia*)

In Chapter 1 of this dissertation, I examine relationships between the adrenocortical stress response, song complexity, and other male quality and fitness metrics in the song sparrow (*Melospiza melodia*). This appendix contains tables reporting non-significant results from statistical tests referenced in the text. Statistics reported are from initial full statistical models.

Table 1.A1. Non-significant fixed effects from linear mixed effects models predicting baseline CORT in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (>F)
Entire dataset				
Repertoire size	0.17 ± 0.23	0.51	63.00	0.47
Syllable number	-0.10 ± 0.08	1.44	63.00	0.24
Stage of capture	0.58 ± 1.05[a]	0.31	63.00	0.58
Time	-0.17 ± 0.13	1.55	63.00	0.22
Early stage				
Repertoire size	0.27 ± 0.29	0.87	49.00	0.35
Syllable number	-0.18 ± 0.11	2.77	49.00	0.10
Time	-0.28 ± 0.18	2.48	49.00	0.12

N = 69 observations, 43 males (entire dataset); N = 54 observations, 43 males (early stage).
[a]Nestling stage relative to early stage.

Table 1.A2. Non-significant fixed effects from linear mixed effects models predicting delta CORT in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Entire dataset				
Date	0.0002 ± 0.02	<0.001	55.93	0.99
Time	-0.08 ± 0.18	0.23	56.00	0.63
Early stage				
Date	0.02 ± 0.03	0.99	42.94	0.33
Time	0.27 ± 0.20	1.76	42.17	0.19

N = 62 observations, 41 males (entire dataset); N = 48 observations, 38 males (early stage).

Table 1.A3. Non-significant fixed effects from linear mixed effects models predicting body condition from song complexity variables in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Entire dataset				
Repertoire size	-0.04 ± 0.10	0.15	35.24	0.70
Syllable diversity	-0.003 ± 0.002	0.15	29.50	0.70
Time of capture	0.01 ± 0.04	0.02	48.96	0.88
Date of capture	0.01 ± 0.01	1.49	57.14	0.23
Early stage				
Repertoire size	0.01 ± 0.10	0.02	38.36	0.89
Syllable diversity	-0.04 ± 0.04	1.10	35.35	0.30
Time of capture	-0.06 ± 0.05	1.60	31.21	0.21
Date of Capture	0.01 ± 0.01	1.34	30.22	0.25

N = 69 observations, 43 males (entire dataset); N = 55 observations, 43 males (early stage).

Table 1.A4. Non-significant fixed effects from linear mixed effects models predicting body condition from CORT variables in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Entire dataset				
Delta CORT	0.03 ± 0.03	0.77	35.24	0.38
Baseline CORT	-0.001 ± 0.002	0.32	29.50	0.57
Time of capture	0.03 ± 0.04	0.52	48.96	0.48
Date of capture	0.01 ± 0.01	1.40	57.14	0.24
Early stage				
Delta CORT	0.001 ± 0.001	0.78	60.73	0.38
Baseline CORT	-0.002 ± 0.002	1.40	17.70	0.25
Time of capture	-0.02 ± 0.05	0.23	35.61	0.64
Date of Capture	0.01 ± 0.01	1.05	43.77	0.31

N = 79 observations, 57 males (entire dataset); N = 66 observations, 55 males (early stage).

Table 1.A5. Non-significant fixed effects from linear mixed effects models predicting hematocrit from song complexity variables in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Entire dataset				
Stage of capture	-0.80 ± 1.19	0.45	65	0.50
Date of capture	0.02 ± 0.02	1.21	65	0.28
Time of capture	0.07 ± 0.15	0.21	65	0.65
Early stage				
Date of capture	0.03 ± 0.02	2.27	50.76	0.14
Time of capture	0.11 ± 0.17	0.42	48.43	0.52

N = 71 observations, 43 males (entire dataset); N = 56 observations, 43 males (early stage).

Table 1.A6. Non-significant fixed effects from linear mixed effects models predicting hematocrit from CORT variables in the entire dataset and early stage

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Entire dataset				
Baseline CORT	0.02 ± 0.01	3.94	50.50	0.053[b]
Stage of capture	0.50 ± 1.15[a]	0.19	62.49	0.66
Date of capture	0.02 ± 0.02	1.12	72.84	0.29
Time of capture	0.04 ± 0.14	0.07	56.16	0.79
Early stage				
Baseline CORT	0.01 ± 0.01	3.08	25.33	0.091[b]
Date of capture	0.03 ± 0.02	1.49	59.33	0.23
Time of capture	0.08 ± 0.17	0.22	51.19	0.64

N = 71 observations, 43 males (entire dataset); N = 67 observations, 56 males (early stage).

[a] Nestling stage relative to early stage.

[b] Marginally significant p-values for baseline CORT became insignificant in reduced models.

Table 1.A7. Non-significant fixed effects from linear models predicting wingchord from song complexity and CORT variables

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Song complexity				
Repertoire size	0.09 ± 0.37	0.93	32	0.54
CORT variables				
delta CORT	-0.0002 ± 0.003	0.04	50	0.85
Baseline CORT	-0.004 ± 0.005	0.56	50	0.46

Table 1.A8. Non-significant fixed effects from generalized linear mixed effects models (Poisson family) predicting nestling number from song complexity and CORT variables

	Estimate (β ± SE)	Z	P (> | z |)
Song complexity			
Repertoire size	-0.17 ± 0.14	-1.18	0.23
Syllable diversity	0.04 ± 0.05	0.85	0.40
CORT variables			
Delta CORT	0.06 ± 0.04	1.40	0.16
Baseline CORT	0.003 ± 0.003	1.09	0.27

N = 41 observations, 34 males (song complexity); N = 58 observations, 46 males (CORT variables).

Table 1.A9. Non-significant fixed effects from linear mixed effects models predicting nestling mass from song complexity and CORT variables

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Song complexity				
Syllable diversity	0.004 ± 0.005	0.52	24.67	0.48
Brood size	-0.004 ± 0.017	0.05	42.47	0.82
CORT variables				
Baseline CORT	-0.0003 ± 0.0004	0.40	30.21	0.53
Brood size	-0.01 ± 0.02	0.33	33.85	0.57

N = 108 observations, 45 nests, 27 males (song complexity); N = 88 observations, 41 nests, 28 males (CORT variables).

Table 1.A10. Non-significant fixed effects from generalized linear mixed effects models (binomial family) predicting survival to the subsequent breeding season from song complexity and CORT variables

	Estimate (β ± SE)	Z	P (> | z |)
Song complexity			
Repertoire size	-0.03 ± 0.04	-0.62	0.53
Syllable diversity	-0.05 ± 0.14	-0.36	0.71
CORT variables			
Delta CORT	-0.05 ± 0.06	-0.80	0.43
Baseline CORT	-0.001 ± 0.004	-0.21	0.84

N = 45 observations, 31 males (song complexity); N =40 observations, 31 males (CORT variables).

APPENDIX 2

CHAPTER 2: Predation risk modifies the relationship between parental effort and song complexity in the song sparrow (*Melospiza melodia*)

In Chapter 2 of this dissertation, I examine the relationship between male song complexity, as measured by song repertoire size and song syllable diversity, and paternal and maternal effort. Further, I examine the extend to which elevated perceived predation risk alters relationships between song complexity and parental behavior. This appendix contains tables that report results for non-significant effects from initial full statistical models referenced in the text.

Table 2.A1. Linear mixed effects models predicting paternal provisioning rate, maternal provisioning rate, and maternal incubation attentiveness from treatment

	Estimate (β ± SE)	t	Denom (df)	P (> | t |)
Paternal offspring provisioning[a]				
Intercept	4.03 ± 0.04	10.20	93	<0.001
Finch v. baseline	0.04 ± 0.57	0.07	93	0.94
Hawk v. baseline	-0.88 ± 0.35	-2.50	93	0.01
Maternal offspring provisioning[a]				
Intercept	2.09 ± 0.11	18.44	93	<0.001
Finch v. baseline	-0.08 ± 0.17	-0.47	93	0.64
Hawk v. baseline	-0.25 ± 0.10	-2.37	93	0.02
Maternal incubation attentiveness[b]				
Intercept	0.49 ± 0.02	24.97	166	<0.001
Finch v. baseline	0.02 ± 0.03	0.51	166	0.61
Jay v. baseline	-0.04 ± 0.02	-1.96	166	0.05

Note: The finch (negative control) and baseline treatments did not significantly differ, whereas the predator and baseline treatments did

[a] N = 136 observations, 49 nests, 38 females, 34 males.

[b] N = 266 observations, 108 nests, 66 females, 52 males.

Table 2.A2. Linear mixed effects model predicting paternal provisioning rates from song traits and predator presence (treatment) showing statistics for non-significant fixed effects

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Treatment	-0.91 ± 0.31[a]	8.74	72.79	0.004
Syllable diversity	-0.50 ± 0.45	0.73	22.40	0.40
Repertoire size	0.57 ± 0.41	3.00	30.70	0.09
Time	0.01 ± 0.08	0.02	87.99	0.89
Brood size	1.07 ± 0.22	23.66	35.50	<0.001
Nestling age	0.63 ± 0.10	42.86	105.17	<0.001
Treatment × repertoire	0.19 ± 0.35	0.28	71.34	0.60
Treatment × syllable	0.28 ± 0.36	0.62	71.35	0.43
Repertoire × syllable	0.20 ± 0.45	0.20	28.27	0.66

[a] Predator (hawk) treatment relative to baseline.

N = 116 observations, 43 nests, 35 males, 30 females.

Table 2.A3. Initial linear mixed effects model predicting male nest attentiveness from song traits and predator presence (treatment) showing statistics for non-significant fixed effects

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Treatment	0.01 ± 0.02[a]	0.06	16.13	0.81
Syllable diversity	-0.02 ± 0.04	2.65	15.59	0.12
Repertoire size	0.07 ± 0.04	6.17	16.64	0.02
Brood size	0.02 ± 0.02	1.07	15.61	0.32
Nestling age	-0.002 ± 0.02	0.005	16.62	0.94
Time	-0.01 ± 0.01	4.37	19.50	0.05[b]
Treatment × repertoire	0.04 ± 0.03	1.58	15.89	0.23
Treatment × syllable	-0.08 ± 0.04	3.35	16.09	0.09
Repertoire × syllable	0.01 ± 0.03	0.08	14.13	0.78

[a] Predator (hawk) treatment relative to baseline.

[b] Became non-significant (*P* = 0.13) upon model reduction.

N = 43 observations, 21 nests, 20 males, 20 females.

Table 2.A4. Linear mixed effects model predicting maternal incubation attentiveness from male song traits and predator presence (treatment) showing statistics for non-significant fixed effects

	Estimate (β± SE)	F	Denom (df)	P (> F)
Treatment	-0.05 ± 0.02[a]	4.39	109.01	0.04
Clutch size	0.01 ± 0.02	0.95	72.54	0.33
Time	-0.01 ± 0.005	6.09	145.97	0.01
Incubation day	-0.01 ± 0.004	3.75	68.14	0.06
Repertoire size	-0.001 ± 0.02	0.02	58.76	0.90
Syllable diversity	0.001 ± 0.35	0.14	40.14	0.71
Treatment × repertoire	-0.002 ± 0.31	0.01	107.62	0.93
Treatment × syllable	0.01 ± 0.31	0.22	108.97	0.64
Repertoire × syllable	0.02 ± 0.35	1.65	55.99	0.20

[a] Predator (hawk) treatment relative to baseline.

N = 188 observations, 77 nests, 41 males, 52 females.

Table 2.A5. Linear mixed effects model predicting maternal nestling stage nest attentiveness from male song traits and predator presence (treatment) showing statistics for non-significant fixed effects

	Estimate (β± SE)	F	Denom (df)	P (> F)
Treatment	-0.23 ± 0.07	10.84	28.99	0.003
Brood size	-0.02 ± 0.04	0.20	28.99	0.66
Nestling age	-0.05 ± 0.04	1.60	28.99	0.22
Time	-0.03 ± 0.02	2.10	28.99	0.15
Repertoire size	0.07 ± 0.08	0.08	28.99	0.77
Syllable diversity	-0.07 ± 0.09	0.58	28.99	0.45
Treatment × repertoire	-0.10 ± 0.09	1.41	28.99	0.24
Treatment × syllable	0.05 ± 0.12	0.17	28.99	0.68
Repertoire × syllable	-0.10 ± 0.06	3.06	28.99	0.09

[a] Predator (hawk) treatment relative to baseline.

N = 39 observations, 21 nests, 20 males, 20 females.

Table 2.A6. Linear mixed effects models predicting percent female provisioning effort from male song traits and predator presence across treatments, in the predation treatment alone, and in the baseline treatment alone showing statistics for non-significant fixed effects

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Across treatments[a]				
Treatment	0.005 ± 0.02[b]	0.07	59.55	0.79
Brood size	-0.02 ± 0.01	2.85	30.76	0.10
Nestling age	0.003 ± 0.01	0.31	87.20	0.58
Time	0.01 ± 0.01	1.59	69.21	0.21
Repertoire size	-0.03 ± 0.02	12.05	30.44	0.002
Syllable diversity	0.04 ± 0.03	8.39	21.51	0.008
Repertoire x treatment	-0.10 ± 0.02	17.96	59.89	<0.001
Syllable x treatment	0.09 ± 0.02	14.29	59.44	<0.001
Repertoire x syllable	0.04 ± 0.03	1.96	22.94	0.18
Within predation treatment[c]				
Repertoire size	-0.09 ± 0.02	18.37	29.48	<0.001
Syllable diversity	0.03 ± 0.01	12.20	21.47	0.002
Brood size	-0.02 ± 0.02	1.39	33.53	0.25
Nestling age	-0.01 ± 0.01	0.60	14.74	0.45
Time	0.003 ± 0.01	0.07	31.34	0.79
Within baseline treatment[d]				
Repertoire size	-0.02 ± 0.02	2.05	29.75	0.16
Syllable diversity	0.01 ± 0.01	1.09	20.97	0.31
Brood size	-0.02 ± 0.01	1.65	30.13	0.21
Nestling age	0.005 ± 0.01	0.62	36.50	0.44
Time	0.004 ± 0.01	0.41	30.40	0.53

[a] N = 100 observations, 41 nests, 35 females, 29 males.

[b] Predator treatment relative to baseline.

[c] N = 44 observations, 38 nests, 33 females, 28 males.

[d] N = 56 observations, 37 nests, 33 females, 26 males.

APPENDIX 3

CHAPTER 3: Variation in parental effort, the adrenocortical stress response, and body condition in the song sparrow (Melospiza melodia)

In Chapter 3 of this dissertation, I examine the relationship between individual-level variation in plasma corticosterone (CORT) levels and parental behaviors in the presence and absence of perceived predation risk. I specifically test the hypothesis that suppression of the adrenocortical stress response may facilitate investment into parental effort, and that predation risk may induce relationships between CORT levels and behavior by elevating the costs of parental care. This appendix includes results pertaining to non-significant correlations referenced in the text. In addition, I include models predicting plasma CORT levels in males and females from noise variables: stage of capture, date of capture, and time of capture. To control for differences in the timing of capture of birds, I took the residuals of regressions relating CORT levels to significant predictors (stage, date, or time) before entry into behavioral models.

Table 3.A1. Linear mixed effects models predicting baseline CORT concentrations from capture stage, time of capture, and residual capture date (controlled for capture stage) in males and females

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Male baseline[a]				
Capture stage	-0.21 ± 0.19	1.24	85.99	0.26
Time of capture	-0.03 ± 0.03	0.56	85.99	0.16
Residual date	-0.01 ± 0.004	2.01	85.99	0.46
Female baseline[b]				
Capture stage	0.14 ± 0.24	0.35	36.19	0.56
Time of capture	-0.06 ± 0.05	1.56	33.56	0.22
Residual date	-0.004 ± 0.006	0.42	13.95	0.53
Male acute[c]				
Capture stage	-0.32 ± 0.12	7.09	54.53	0.01
Time of capture	-0.05 ± 0.02	4.36	75.70	0.04[d]
Residual date	-0.004 ± 0.003	1.92	79.97	0.17
Female acute[e]				
Capture stage	-0.19 ± 0.11	2.83	10.56	0.12
Time of capture	-0.08 ± 0.03	8.89	27.55	0.006
Residual date	-0.009 ± 0.003	13.32	7.52	0.007
Male delta[f]				
Capture stage	-2.14 ± 1.08	3.89	72.88	0.052
Time of capture	-0.16 ± 0.19	0.72	76.96	0.40
Residual date	0.002 ± 0.02	0.005	76.00	0.94
Female delta[g]				
Capture stage	-1.27 ± 0.79	2.55	29.07	0.12
Time of capture	-0.46 ± 0.17	7.55	30.89	0.01
Residual date	-0.04 ± 0.02	3.15	21.54	0.09

[a] N = 90 observations, 63 males.

[b] N = 42 observations, 36 females.

[c] N = 84 observations, 59 males.

[d] Became non-significant in step two of model reduction ($F_{1,77} = 3.38$, $\beta = -0.04 \pm 0.02$, $P = 0.07$).

[e] N = 38 observations, 33 females.

[f] N = 81 observations, 59 males.

[g] N = 35 observations, 31 females.

Table 3.A2. Linear mixed effects models predicting paternal provisioning rate, maternal provisioning rate, and maternal incubation attentiveness from treatment

	Estimate (β ± SE)	t	Denom (df)	P (> | t |)
Paternal offspring provisioning[a]				
Intercept	4.03 ± 0.04	10.20	93	<0.001
Finch v. baseline	0.04 ± 0.57	0.07	93	0.94
Hawk v. baseline	-0.88 ± 0.35	-2.50	93	0.01
Maternal offspring provisioning[a]				
Intercept	2.09 ± 0.11	18.44	93	<0.001
Finch v. baseline	-0.08 ± 0.17	-0.47	93	0.64
Hawk v. baseline	-0.25 ± 0.10	-2.37	93	0.02
Maternal incubation attentiveness[b]				
Intercept	0.49 ± 0.02	24.97	166	<0.001
Finch v. baseline	0.02 ± 0.03	0.51	166	0.61
Jay v. baseline	-0.04 ± 0.02	-1.96	166	0.05

Note: The finch (negative control) and baseline treatments did not significantly differ, whereas the predator and baseline treatments did.

[a] N = 136 observations, 49 nests, 38 females, 34 males.

[b] N = 266 observations, 108 nests, 66 females, 52 males.

Table 3.A3. Linear mixed effects models predicting female incubation attentiveness from treatment and (in separate models) delta CORT (residuals), acute CORT (residuals), baseline CORT, and body condition

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Delta CORT [a]				
Treatment	-0.05 ± 0.03[b]	3.70	64.72	0.05
Delta CORT	-0.01 ± 0.02	0.03	39.03	0.88
Incubation day	-0.01 ± 0.005	2.89	40.84	0.09
Time	-0.001 ± 0.01	0.005	93.47	0.94
Date	0.001 ± 0.001	0.28	31.13	0.59
Clutch size	0.02 ± 0.02	0.60	42.59	0.44
Treatment × delta	0.04 ± 0.02	3.19	63.64	0.08
Acute CORT[c]				
Treatment	-0.06 ± 0.03 [b]	4.01	67.68	0.04
Acute CORT	-0.005 ± 0.03	0.02	40.30	0.89
Incubation day	-0.01 ± 0.004	4.14	43.08	0.04
Time	-0.002 ± 0.01	0.08	98.82	0.77
Date	0.001 ± 0.001	0.19	33.25	0.66
Clutch size	0.01 ± 0.02	0.44	46.08	0.51
Treatment × acute	0.02 ±0.04	0.21	66.87	0.65
Baseline CORT[d]				
Treatment	-0.03 ± 0.05 [b]	0.53	70.62	0.47
Baseline CORT	0.001 ± 0.005	0.15	37.05	0.70
Incubation day	-0.008 ± 0.004	3.15	42.42	0.08
Time	-0.01 ± 0.007	2.34	99.16	0.12
Date	0.0003 ± 0.001	0.09	35.94	0.76
Clutch size	0.02 ± 0.02	0.71	45.11	0.40
Treatment × baseline	-0.001 ± 0.001	0.01	72.13	0.49
Condition[e]				
Treatment	-0.08 ± 0.03[b]	8.67	79.12	0.004
Condition	-0.01 ± 0.01	1.16	55.77	0.29
Incubation day	-0.01 ± 0.004	6.86	55.14	0.01
Time	-0.01 ± 0.007	1.75	115.58	0.19
Date	0.001 ± 0.001	0.65	53.05	0.42
Clutch size	0.02 ± 0.02	1.33	58.83	0.25
Treatment × condition	-0.005 ± 0.02	0.08	79.98	0.77

[a] N = 114 observations, 48 nests, 30 females, 29 males.

[b] Predator (jay) treatment relative to baseline.

[c] N = 119 observations, 50 nests, 32 females, 30 males.

[d] N = 125 observations, 54 nests, 34 females, 33 males.

[e] N = 139 observations, 60 nests, 37 females, 35 males.

Table 3.A4. Linear mixed effects models predicting maternal provisioning rates from treatment and (in separate models) delta CORT (residuals), acute CORT (residuals), baseline CORT, and body condition

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Delta CORT [a]				
Treatment	-0.18 ± 0.08[b]	4.53	35.63	0.04
Delta CORT	0.007 ± 0.09	0.09	21.59	0.76
Nestling age	0.15 ± 0.03	24.36	49.05	<0.001
Time	0.003 ± 0.03	0.02	41.60	0.88
Date	-0.001 ± 0.004	0.04	19.68	0.85
Brood size	0.25 ± 0.10	6.52	23.45	0.02
Treatment × delta	-0.06 ± 0.07	0.83	34.12	0.37
Acute CORT[c]				
Treatment	-0.16 ± 0.09 [b]	2.99	36.57	0.09
Acute CORT	0.04 ± 0.15	0.26	23.25	0.61
Nestling age	0.14 ± 0.03	24.14	52.64	<0.001
Time	-0.002 ± 0.03	0.01	43.62	0.93
Date	-0.001 ± 0.004	0.02	21.10	0.87
Brood size	0.28 ± 0.09	9.37	24.63	0.005
Treatment × acute	0.06 ± 0.13	0.24	35.57	0.62
Baseline CORT[d]				
Treatment	-0.17 ± 0.08 [b]	4.91	48.45	0.03
Baseline CORT	-0.16 ± 0.10	2.99	22.71	0.09
Nestling age	0.17 ± 0.02	52.37	66.99	<0.001
Time	0.02 ± 0.02	0.51	58.44	0.48
Date	-0.0003 ± 0.005	0.01	23.55	0.93
Brood size	0.22 ± 0.07	11.14	24.25	0.003
Treatment × baseline	-0.03 ± 0.08	0.15	47.91	0.70
Condition[e]				
Treatment	-0.14 ± 0.08[b]	3.38	50.71	0.07
Condition	-0.26 ± 0.19	3.33	27.91	0.08
Nestling age	0.17 ± 0.02	52.98	72.20	<0.001
Time	0.02 ± 0.02	0.66	61.69	0.42
Date	-0.001 ± 0.004	0.03	25.16	0.87
Brood size	0.20 ± 0.06	11.11	26.41	0.003
Treatment × condition	-0.12 ± 0.15	0.61	49.22	0.44

[a] N = 60 observations, 24 nests, 18 females, 19 males.
[b] Predator (hawk) treatment relative to baseline.
[c] N = 62 observations, 25 nests, 19 females, 20 males.
[d] N = 77 observations, 29 nests, 21 females, 24 males.
[e] N = 81 observations, 31 nests, 23 females, 25 males.

Table 3.A5. Linear mixed effects models predicting maternal nestling stage nest attentiveness from treatment and (in separate models) delta CORT (residuals), acute CORT (residuals), baseline CORT, and body condition

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Delta CORT [a]				
Treatment	-0.26 ± 0.08[b]	11.89	4.31	0.02
Delta CORT	-0.05 ± 0.04	3.47	3.48	0.07
Nestling age	-0.13 ± 0.04	9.99	3.66	0.04
Time	-0.06 ± 0.03	6.00	9.45	0.04
Date	-0.001 ± 0.003	0.04	3.16	0.85
Brood size	-0.04 ± 0.07	0.45	5.03	0.53
Treatment × delta	-0.08 ± 0.05	3.22	3.39	0.16
Acute CORT[c]				
Treatment	-0.26 ± 0.08 [b]	11.08	5.86	0.02
Acute CORT	-0.12 ± 0.07	10.49	4.26	0.03
Nestling age	-0.15 ± 0.04	17.18	3.94	0.01
Time	-0.07 ± 0.03	6.85	12.86	0.06
Date	0.004 ± 0.003	1.68	3.42	0.02
Brood size	-0.03 ± 0.06	0.21	5.34	0.67
Treatment × acute	-0.14 ± 0.09	2.41	4.77	0.18
Baseline CORT[d]				
Treatment	-0.36 ± 0.06 [b]	29.99	7.31	0.001
Baseline CORT	0.001 ± 0.12	0.46	8.89	0.51
Nestling age	-0.13 ± 0.06	5.66	9.32	0.01
Time	-0.08 ± 0.02	10.09	12.06	0.01
Date	0.002 ± 0.005	0.21	8.27	0.65
Brood size	-0.03 ± 0.08	0.19	8.99	0.68
Treatment × baseline	-0.16 ± 0.10	2.43	7.44	0.16
Condition[e]				
Treatment	-0.35 ± 0.08[b]	21.18	7.97	0.002
Condition	0.18 ± 0.12	2.07	7.64	0.19
Nestling age	-0.11 ± 0.05	4.63	8.22	0.06
Time	-0.06 ± 0.03	5.54	14.71	0.03
Date	-0.0002 ± 0.004	0.002	7.69	0.97
Brood size	-0.01 ± 0.08	0.007	8.86	0.94
Treatment × condition	-0.05 ± 0.11	0.23	7.96	0.64

[a] N = 22 observations, 13 nests, 12 females, 12 males.

[b] Predator (hawk) treatment relative to baseline.

[c] N = 22 observations, 13 nests, 12 females, 12 males.

[d] N = 25 observations, 15 nests, 14 females, 14 males.

[e] N = 81 observations, 31 nests, 23 females, 25 males.

Table 3.A6. Linear mixed effects models predicting paternal provisioning rate from treatment and (in separate models) baseline CORT and body condition

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Treatment	-0.23 ± 0.07[b]	10.28	67.47	0.002
Baseline CORT	0.03 ± 0.09	0.15	33.94	0.70
Nestling age	0.09 ± 0.02	19.45	102.18	<0.001
Time	-0.002 ± 0.02	0.02	81.89	0.89
Date	0.004 ± 0.004	1.12	33.48	0.30
Brood size	0.26 ± 0.02	17.17	33.44	<0.001
Treatment × baseline	-0.004 ± 0.07	0.004	66.41	0.95
Condition[c]				
Treatment	-0.25 ± 0.07 [b]	11.93	64.99	<0.001
Condition	0.04 ± 0.30	0.03	33.08	0.87
Nestling age	0.09 ± 0.02	20.06	98.44	<0.001
Time	-0.0001 ± 0.004	0	78.89	0.99
Date	0.003 ± 0.004	0.71	30.99	0.41
Brood size	0.27 ± 0.06	16.83	31.57	<0.001
Treatment × Condition	-0.17 ± 0.24	0.47	62.76	0.49

[a] N = 11 observations, 41 nests, 31 males, 31 females.

[b] Predator (hawk) treatment relative to baseline.

[c] N = 107 observations, 39 nests, 29 males, 30 females.

Table 3.A7. Linear mixed effects models predicting paternal nestling stage nest attentiveness from treatment and (in separate models) delta CORT (residuals), acute CORT (residuals), baseline CORT, and body condition

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Delta CORT [a]				
Treatment	0.02 ± 0.02[b]	0.87	13.70	0.36
Delta CORT	0.01 ± 0.008	7.28	14.11	0.01
Nestling age	-0.04 ± 0.01	12.11	13.64	0.003
Time	-0.003 ± 0.01	0.16	17.21	0.69
Date	0.003 ± 0.001	7.25	12.88	0.02
Brood size	0.02 ± 0.02	0.86	12.41	0.37
Treatment × delta	0.006 ± 0.009	0.50	13.87	0.49
Acute CORT[c]				
Treatment	0.04 ± 0.02 [b]	3.03	12.83	0.11
Acute CORT	0.02 ± 0.09	1.17	13.56	0.30
Nestling age	-0.04 ± 0.02	8.59	15.58	0.01
Time	-0.01 ± 0.006	1.73	15.39	0.21
Date	0.003 ± 0.003	2.59	14.05	0.13
Brood size	0.03 ± 0.03	1.11	13.74	0.31
Treatment × acute	0.15 ± 0.07	4.74	12.30	0.05
Baseline CORT[d]				
Treatment	0.00001 ± 0.03[b]	0	15.83	0.99
Baseline CORT	0.003 ± 0.02	0.004	14.46	0.95
Nestling age	-0.04 ± 0.02	5.61	16.39	0.03
Time	-0.003 ± 0.01	0.16	22.10	0.69
Date	0.002 ± 0.001	2.44	15.12	0.14
Brood size	0.02 ± 0.02	0.46	15.05	0.51
Treatment × baseline	-0.002 ± 0.02	0.01	16.68	0.92
Condition[e]				
Treatment	0.02 ± 0.02[b]	0.38	13.37	0.55
Condition	-0.05 ± 0.06	1.37	12.06	0.26
Nestling age	-0.04 ± 0.01	8.37	14.32	0.01
Time	-0.006 ± 0.008	0.84	16.61	0.37
Date	0.003 ± 0.002	0.84	13.21	0.08
Brood size	0.006 ± 0.02	0.07	12.51	0.80
Treatment × condition	-0.02 ± 0.06	0.21	12.06	0.66

[a]N = 34 observations, 19 nests, 18 males, 18 females.
[b]Predator (hawk) treatment relative to baseline.
[c]N = 34 observations, 19 nests, 18 males, 18 females.
[d]N = 38 observations, 21 nests, 20 males, 20 females.
[e]N = 34 observations, 19 nests, 18 males, 18 females.

APPENDIX 4

CHAPTER 4: Sex-specific modulation of the adrenocortical stress response, parental effort and parental risk-taking behavior in the song sparrow (*Melospiza melodia*)

In Chapter 4 of this dissertation, I examine sex differences in corticosterone (CORT) levels and body condition across the breeding season in the song sparrow. Specifically, I test the predictions that females, which have higher certainty of parentage and alone incubate eggs, display lower CORT levels than males, and that the stress response is suppressed in both sexes during the parentally demanding nestling stage. In addition, I examine whether males and females display differences in parental effort and risk-taking that are consistent with theory based on differences in CORT levels. This appendix contains tables that report statistics from initial full statistical models for non-significant results referenced in the main text.

Table 4.A1. Linear mixed effect models predicting baseline, delta, and acute CORT from sex, capture stage, residual date, and time of capture

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Intercept	3.39 ± 0.38	---	---	---
Sex[b]	0.93 ± 0.21	24.14	124.98	<0.001
Capture stage[c]	0.15 ± 0.23	0.06	124.98	0.81
Residual date	-0.005 ± 0.006	2.09	124.98	0.15
Time	-0.04 ± 0.03	1.53	124.98	0.22
Sex × stage	-0.38 ± 0.30	1.61	124.98	0.20
Sex × date	-0.001 ± 0.01	0.03	124.98	0.86
Delta CORT[d]				
Intercept	11.37 ± 1.97	---	---	---
Sex[b]	2.98 ± 1.22	9.54	102.06	0.003
Capture stage[c]	-1.28 ± 1.33	4.31	106.39	0.04
Residual date	-0.03 ± 0.03	0.66	104.88	0.42
Time	-0.24 ± 0.15	2.84	109.00	0.09
Sex × stage	-0.86 ± 1.65	0.27	106.39	0.60
Sex × date	0.03 ± 0.04	0.61	103.96	0.43
Acute CORT[e]				
Intercept	5.19 ± 0.25	---	---	---
Sex[b]	0.70 ± 0.15	32.33	107.47	<0.001
Capture stage[c]	-0.16 ± 0.16	6.30	93.82	0.01
Residual date	-0.008 ± 0.004	6.60	101.50	0.01
Time	-0.06 ± 0.02	10.59	109.01	0.002
Sex × stage	-0.17 ± 0.20	0.79	94.61	0.38
Sex × date	0.004 ± 0.005	0.51	98.83	0.48

[a] N = 132 observations, 99 individuals.

[b] Males relative to females.

[c] Nestling stage relative to early stage.

[d] N = 116 observations, 90 individuals.

[e] N = 122 observations, 92 individuals.

Table 4.A2. Linear mixed effect models predicting baseline, delta, and acute CORT from individual sex, capture date and time of capture within early-stage captures

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Intercept	3.76 ± 0.46	---	---	---
Sex[b]	0.90 ± 0.21	17.91	80.99	<0.001
Date	0.07 ± 0.27	0.09	80.99	0.76
Time	-0.07 ± 0.04	3.35	80.99	0.07
Sex × date	-0.21 ± 0.28	0.60	80.99	0.44
Delta CORT[c]				
Intercept	9.81 ± 2.47	---	---	---
Sex[a]	2.28 ± 1.43	2.56	69.32	0.11
Date	1.46 ± 1.83	0.90	69.66	0.35
Time	-0.04 ± 0.19	0.05	68.57	0.82
Sex × date	-1.13 ± 1.88	0.36	69.71	0.55
Acute CORT[d]				
Intercept	5.05 ± 0.29	---	---	---
Sex[a]	0.59 ± 0.17	12.88	70.46	<0.001
Date	0.24 ± 0.21	0.94	71.55	0.34
Time	-0.03 ± 0.02	2.52	69.21	0.12
Sex × date	-0.27 ± 0.22	1.49	71.59	0.23

[a] N = 86 observation, 75 individuals.

[b] Males relative to females.

[c] N = 75 observations, 65 individuals.

Table 4.A3. Linear mixed effect models predicting baseline, delta, and acute CORT from individual sex, capture date and time of capture within nestling stage captures

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Intercept	3.02 ± 0.51	---	---	---
Sex[b]	0.54 ± 0.22	6.14	35.83	0.02
Date	-0.11 ± 0.11	0.12	38.24	0.73
Time	0.01 ± 0.05	0.07	33.72	0.79
Sex × date	0.15 ± 0.22	0.48	38.27	0.49
Delta CORT[c]				
Intercept	13.60 ± 2.19	---	---	---
Sex[b]	2.18 ± 0.86	6.39	36.00	0.01
Date	-0.82 ± 0.48	8.27	36.00	0.01
Time	-0.56 ± 0.19	8.49	36.00	0.01
Sex × date	-1.18 ± 0.98	1.46	36.00	0.23
Acute CORT[d]				
Intercept		---	---	---
Sex[b]	0.56 ± 0.14	16.54	34.05	<0.001
Date	-0.19 ± 0.05	13.14	35.85	0.001
Time	-0.12 ± 0.02	23.03	4.72	0.006
Sex × date	-0.13 ± 0.14	0.82	34.13	0.36

[a] N = 46 observations, 41 individuals.

[b] Males relative to females.

[c] N = 41 observations, 38 individuals.

[d] N = 43 observations, 40 individuals.

Table 4.A4. Linear mixed effect model predicting body condition from sex, capture date and time of capture within nestling stage captures

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Intercept	0.44 ± 0.79	---	---	---
Sex[a]	0.02 ± 0.43	0.002	32.87	0.96
Date	0.25 ± 0.16	1.13	35.99	0.30
Time	-0.11 ± 0.07	2.26	24.00	0.15
Sex × date	-0.02 ± 0.45	0.002	35.99	0.96

[a] Males relative to females.

N = 41 observations, 36 individuals.

Table 4.A5. Linear mixed effect models predicting baseline, delta and acute CORT and body condition from stage of capture (early versus nestling), capture date and time of capture in males

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Intercept	4.21 ± 0.38	---	---	---
Capture stage[b]	-0.21 ± 0.19	1.24	85.99	0.27
Date	-0.01 ± 0.004	2.01	85.99	0.16
Time	-0.03 ± 0.03	0.56	85.99	0.46
Delta CORT[c]				
Intercept	13.44 ± 2.18	---	---	---
Capture stage[b]	-2.14 ± 1.08	3.89	72.88	0.05
Date	0.002 ± 0.03	0.005	76.00	0.94
Time	-0.16 ± 0.19	0.73	76.96	0.40
Acute CORT[d]				
Intercept	5.75 ± 0.25	---	---	---
Capture stage[b]	-0.32 ± 0.12	7.09	54.52	0.01
Date	-0.004 ± 0.003	1.92	79.97	0.17
Time	-0.04 ± 0.02	4.36	75.70	0.04
Body condition[e]				
Intercept	0.003 ± 0.54	---	---	---
Capture stage[b]	-0.87 ± 0.27	10.47	31.37	0.003
Date	0.008 ± 0.007	1.64	73.82	0.20
Time	0.01 ± 0.05	0.04	58.46	0.84

[a] N = 90 observations, 63 individuals.

[b] Nestling stage relative to early stage.

[c] N = 81 observations, 59 individuals.

[d] N = 84 observations, 59 individuals.

[e] N = 82 observations, 59 individuals.

Table 4.A6. Linear mixed effect models predicting baseline, delta and acute CORT and body condition from stage of capture (early versus nestling), capture date and time of capture in females

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Baseline CORT[a]				
Intercept	3.70 ± 0.62	---	---	---
Capture stage[b]	0.14 ± 0.24	0.35	36.19	0.57
Date	-0.004 ± 0.006	0.42	13.95	0.53
Time	-0.06 ± 0.05	1.56	33.56	0.22
Delta CORT[c]				
Intercept	13.64 ± 1.95	---	---	---
Capture stage[b]	-1.27 ± 0.79	2.55	29.07	0.12
Date	-0.03 ± 0.02	3.16	21.54	0.09
Time	-0.46 ± 0.17	7.55	30.89	0.01
Acute CORT[d]				
Intercept	5.48 ± 0.34	---	---	---
Capture stage[b]	-0.19 ± 0.11	2.83	10.56	0.01
Date	-0.01 ± 0.003	13.32	13.32	0.12
Time	-0.08 ± 0.03	8.79	27.55	0.01
Body condition[e]				
Intercept	3.28 ± 1.13	---	---	---
Capture stage[b]	-2.13 ± 0.44	23.36	34.76	<0.001
Date	0.02 ± 0.01	4.32	15.19	0.054
Time	-0.18 ± 0.09	3.58	30.56	0.068

[a] N = 42 observations, 36 individuals.

[b] Nestling stage relative to early stage.

[c] N = 35 observations, 31 individuals.

[d] N = 38 observations, 33 individuals.

[e] N = 43 observations, 37 individuals.

APPENDIX 5

CHAPTER 5: Phenotypic plasticity in nest departure calls: weighing costs and benefits

In Chapter 5 of this dissertation, I examine whether production of a unique female vocalization, the nest departure calls (NDC), in song sparrows (*Melospiza melodia*) varies as a function of factors that may affect the costs and benefits of calling including male presence, male quality (as measured by song complexity and body condition), nest predator presence, and nest height. In addition, I examine whether male nest-guarding or female incubation behavior is predicted by nest departure calling, while also considering the effects of the other variables noted above. Here I report statistics for non-significant effects referenced in the text. Statistics are reported from the initial whole models.

Table 5.A1. Statistics for non-significant fixed effects from a generalized linear mixed effects model (binomial family) predicting NDC production

	Estimate (β ± SE)	Z	P (> | z |)
Repertoire size	-0.33 ± 0.65	-0.51	0.61
Syllable diversity	0.40 ± 0.63	0.63	0.53
Date	0.003 ± 0.03	0.12	0.91
Repertoire × treatment	0.34 ± 0.55	0.63	0.53
Syllable × treatment	-0.30 ± 0.59	-0.50	0.61
Male present × treatment	-0.13 ± 1.20	-0.11	0.92
Male condition × treatment	-0.45 ± 0.68	-0.67	0.51

N = 330 nest departures, 47 nests, 38 females, 31 males.

Table 5.A2. Statistics for non-significant fixed effects from a generalized linear mixed effects model (binomial family) predicting male nest guarding

	Estimate (β ± SE)	Z	P (> | z |)
Treatment	0.46 ± 0.65[a]	0.71	0.48
Male condition	-0.23 ± 0.43	-0.52	0.60
Nest height	0.38 ± 0.85	0.45	0.65
Repertoire size	0.45 ± 0.45	1.01	0.31
Syllable diversity	-0.42 ± 0.46	-0.92	0.36
Time	<0.001 ± 0.001	0.39	0.70
Date	<0.001 ± 0.02	0.005	0.995
NDC × treatment	-0.70 ± 0.85	-0.83	0.41
Male condition × treatment	0.73 ± 0.47	1.55	0.12
Nest height × treatment	-1.25 ± 0.80	-1.57	0.12
Repertoire × treatment	-0.14 ± 0.38	-0.38	0.70
Syllable × treatment	0.10 ± 0.39	0.26	0.80

[a] Predator (jay) treatment relative to baseline.

N = 330 nest departures, 47 nests, 38 females, 31 males.

Table 5.A3. Statistics for non-significant fixed effects from a linear mixed effects model predicting incubation off-bout length

	Estimate (β ± SE)	F	Df (Denom	P (> F)
NDC	0.12 ± 0.09	3.47	164.92	0.06
Treatment	-0.09 ± 0.07[a]	1.52	282.00	0.22
Male condition	0.09 ± 0.07	2.01	50.50	0.16
Repertoire size	-0.01 ± 0.06	0.04	25.86	0.84
Syllable diversity	-0.07 ± 0.07	0.96	23.71	0.34
Date	0.001 0.002	0.17	22.27	0.68
NDC × treatment	0.03 ± 0.13	0.05	284.64	0.83
NDC × male condition	-0.09 ± 0.09	1.14	196.45	0.29
NDC × repertoire	0.02 ± 0.08	0.04	157.58	0.85
NDC × syllable	-0.04 ± 0.08	0.26	81.07	0.61

Note: The marginally significant effect of NDC production becomes weaker in a reduced model ($F_{1, 269} = 2.27$, $P = 0.13$).

[a] Predator (jay) treatment relative to baseline.

N = 306 nest departures, 47 nests, 38 females, 31 males.

Table 5.A4. Statistics for non-significant fixed effects from a linear mixed effects model predicting incubation attentiveness

	Estimate (β ± SE)	F	Denom (df)	P (> F)
Percent NDC	-0.04 ± 0.05	0.52	57.74	0.47
Treatment	-0.05 ± 0.04[a]	1.94	55.03	0.16
Repertoire size	-0.15 ± 0.01	1.55	33.10	0.22
Syllable diversity	-0.67 ± 0.01	0.14	33.50	0.71
Date	-0.33 ± 0.001	0.40	33.77	0.53
Time	-0.41 ± 0.01	1.33	75.42	0.25

[a] Predator (jay) treatment relative to baseline.

N = 84 observations, 45 nests, 35 females, 31 males.

APPENDIX 6

CHAPTER 6: Variation in adrenocortical stress physiology and condition-related metrics within a heterogeneous urban environment

In Chapter 6 of this dissertation, I investigate differences in adrenocortical stress physiology, body condition, and total antioxidant capacity between song sparrows (*Melospiza melodia*) breeding within a heterogeneous urban environment characterized by activity centers (high disturbance levels) and refuges (buffered from disturbance). This appendix provides statistical details regarding original full models and non-significant results referenced in the text.

Table 6.A1. Original linear mixed effects (entire dataset) and linear (subset data) models predicting plasma CORT levels from habitat type and covariates

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset					
Acute CORT (ng/ml)	83, 78[a]				
Intercept		5.36 ± 0.40	---	---	---
Habitat type		-0.19 ± 0.12[b]	2.75	75.24	0.10
Time of capture		-0.03 ± 0.03	0.65	51.91	0.42
Date of capture		-0.006 ± 0.004	2.49	34.75	0.12
Baseline CORT (ng/ml)	87, 82				
Intercept		5.15 ± 0.55	---	---	---
Habitat type		-0.03 ± 0.16	0.03	80.36	0.85
Time of capture		-0.14 ± 0.05	8.74	80.14	0.004
Date of capture		-0.01 ± 0.005	3.33	74.88	0.07
Delta CORT (ng/ml)	82, 77				
Intercept		4.50 ± 0.48	---	---	---
Habitat type		-0.27 ± 0.14	3.82	77.99	0.05
Time of capture		0.02 ± 0.04	0.36	77.99	0.55
Date of capture		-0.01 ± 0.005	1.67	77.99	0.20
Subset data					
Acute CORT (ng/ml)	50				
Intercept		5.26 ± 0.58	---	---	---
Habitat type		-0.27 ± 0.15	3.20	46	0.08
Time of capture		-0.02 ± 0.05	0.26	46	0.62
Date of capture		-0.01 ± 0.01	0.63	46	0.43
Baseline CORT (ng/ml)	50				
Intercept		4.78 ± 0.76	---	---	---
Habitat type		-0.12 ± 0.19	0.11	46	0.74
Time of capture		-0.13 ± 0.06	4.37	46	0.04
Date of capture		-0.001 ± 0.01	0.02	46	0.88
Delta CORT (ng/ml)	50				
Intercept		4.60 ± 0.68	---	---	---
Habitat type		-0.33 ± 0.17	3.94	46	0.053
Time of capture		0.02 ± 0.06	0.04	46	0.84
Date of capture		-0.01 ± 0.01	0.70	46	0.41

[a] Number of observations, number of unique individuals.

[b] Activity center contrasted to activity refuge habitat.

Table 6.A2. Original linear mixed effects models predicting physiological condition metrics from habitat type and plasma CORT variables in the entire dataset

	N	Estimate (β ± SE)	F	Denom (df)	P
Body mass (g)	80, 75[a]				
Intercept		19.54 ± 0.71	---	---	---
Habitat type		0.29 ± 0.25[b]	1.34	71.34	0.25
Delta CORT		-0.03 ± 0.15	0.30	33.53	0.59
Baseline CORT		-0.30 ± 0.31	1.05	70.31	0.31
Time of capture		0.05 ± 0.06	0.67	14.46	0.43
Date of capture		-0.002 ± 0.006	0.12	7.26	0.74
Habitat × delta CORT		-0.04 ± 0.19	0.05	58.95	0.83
Habitat × baseline CORT		0.27 ± 0.33	0.71	71.21	0.40
Hematocrit (%)	82, 77				
Intercept		52.91 ± 3.77	---	---	---
Habitat type		0.22 ± 1.08	0.04	73.99	0.84
Delta CORT		0.90 ± 0.71	0.59	73.99	0.44
Baseline CORT		1.03 ± 1.28	0.33	73.99	0.57
Time of capture		-0.32 ± 0.32	1.00	73.99	0.32
Date of capture		-0.02 ± 0.04	0.24	73.99	0.62
Habitat × delta CORT		-1.08 ± 0.93	1.33	73.99	0.25
Habitat × baseline CORT		-1.26 ± 1.38	0.83	73.99	0.37
Feather PC1					
Intercept	82, 77	-0.33 ± 0.27	---	---	---
Habitat type		0.52 ± 0.32	2.63	66.50	0.11
Delta CORT		-0.06 ± 0.21	0.0001	75.91	0.99
Baseline CORT		0.22 ± 0.40	0.17	66.01	0.68
Habitat × delta CORT		0.12 ± 0.28	0.18	75.91	0.67
Habitat × baseline CORT		-0.28 ± 0.43	0.42	66.01	0.52
TAC (mM HOCl)	52				
Intercept		5.71 ± 0.05	---	---	---
Habitat type		-0.04 ± 0.06[b]	0.61	44	0.44
Delta CORT		-0.02 ± 0.04	0.60	44	0.44
Baseline CORT		0.01 ± 0.07	6.24	44	0.02
Time of capture		0.04 ± 0.03	0.66	44	0.42
Date of capture		0.01 ± 0.03	0.008	44	0.93
Habitat × delta CORT		-0.01 ± 0.06	0.05	44	0.83
Habitat × baseline CORT		0.07 ± 0.08	1.01	44	0.32

[a] Number of observations, number of unique individuals.

[b] Activity center contrasted to activity refuge habitat.

Table 6.A3. Original linear models predicting physiological condition metrics from habitat type and plasma CORT variables in the subset data

	N	Estimate (β ± SE)	F	Denom (df)	P
Body mass (g)	49				
Intercept		19.24 ± 1.10	---	---	---
Habitat type		0.36 ± 0.27[a]	1.73	41	0.20
Delta CORT		-0.25 ± 0.25	2.14	41	0.15
Baseline CORT		-0.05 ± 0.18	0.07	41	0.79
Time		0.08 ± 0.09	0.52	41	0.43
Date		0.0003 ± 0.01	<0.001	41	0.96
Habitat × delta CORT		0.14 ± 0.26	0.30	41	0.65
Habitat × baseline CORT		-0.001 ± 0.27	<0.001	41	0.99
Hematocrit (%)	49				
Intercept		58.93 ± 4.89			
Habitat type		1.35 ± 1.19	2.38	41	0.13
Delta CORT		1.12 ± 0.83	0.005	41	0.24
Baseline CORT		0.73 ± 1.13	1.43	41	0.94
Time of capture		-0.76 ± 0.40	2.90	41	0.09
Date of capture		-0.06 ± 0.06	0.91	41	0.35
Habitat × delta CORT		-2.18 ± 1.21	3.23	41	0.85
Habitat × baseline CORT		-0.37 ± 1.34	0.04	41	0.08
Feather PC1	50				
Intercept		-0.51 ± 0.22	---	---	---
Habitat type		0.17 ± 0.31	0.53	44	0.47
Delta CORT		0.03 ± 0.21	0.84	44	0.37
Baseline CORT		0.11 ± 0.30	3.22	44	0.08
Habitat × delta CORT		-0.30 ± 0.31	0.92	44	0.34
Habitat × baseline CORT		-0.49 ± 0.34	1.91	44	0.17

[a]Activity center contrasted to activity refuge habitat.

Table 6.A4. Linear mixed effects and linear (subset data) models predicting acute CORT concentrations from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	83, 78[a]				
Intercept		5.27 ± 0.38	---	---	---
Noise PC1		0.06 ± 0.04	2.72	76.07	0.10
Noise PC2		-0.02 ± 0.05	0.17	69.07	0.68
Time of capture		-0.03 ± 0.03	0.76	51.87	0.39
Date of capture		-0.01 ± 0.004	2.92	33.70	0.10
Subset data	50				
Intercept		5.22 ± 0.64	---	---	---
Noise PC1		0.05 ± 0.04	1.11	45	0.30
Noise PC2		-0.03 ± 0.08	0.01	45	0.91
Time of capture		-0.03 ± 0.05	0.26	45	0.62
Date of capture		-0.01 ± 0.01	0.75	45	0.39
Activity center only	58, 54				
Intercept		5.71 ± 0.49	---	---	---
Noise PC1		0.08 ± 0.07	0.26	53	0.61
Noise PC2		-0.006 ± 0.05	0.04	53	0.84
Time of capture		-0.07 ± 0.05	3.79	53	0.06
Date of capture		-0.01 ± 0.004	3.63	53	0.06

[a] Number of observations, number of unique individuals.

Table 6.A5. Linear mixed effects and linear (subset data) models predicting delta CORT from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	82, 77[a]				
Intercept		4.33 ± 0.47	---	---	---
Noise PC1		0.06 ± 0.04	2.14	76.99	0.14
Noise PC2		-0.02 ± 0.05	0.17	76.99	0.68
Time of capture		0.02 ± 0.04	0.28	76.99	0.60
Date of capture		-0.01 ± 0.01	1.477	76.99	0.23
Subset data	50				
Intercept		4.48 ± 0.75	---	---	---
Noise PC1		0.06 ± 0.05	1.27	45	0.27
Noise PC2		-0.02 ± 0.09	0.001	45	0.97
Time of capture		0.01 ± 0.06	0.04	45	0.84
Date of capture		-0.01 ± 0.01	0.71	45	0.40
Activity center only	57, 53				
Intercept		4.75 ± 0.60	---	---	---
Noise PC1		0.03 ± 0.08	0.09	52	0.76
Noise PC2		0.001 ± 0.06	0.0001	52	0.99
Time of capture		-0.02 ± 0.06	0.17	52	0.68
Date of capture		-0.01 ± 0.01	0.84	52	0.36

[a] Number of observations, number of unique individuals.

Table 6.A6. Linear mixed effects and linear (subset data) models predicting baseline CORT from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	87, 82[a]				
Intercept		5.24 ± 0.53	---	---	---
Noise PC1		0.06 ± 0.05	1.43	79.96	0.23
Noise PC2		0.04 ± 0.06	0.32	73.68	0.57
Time of capture		-0.14 ± 0.05	9.12	78.49	0.003
Date of capture		-0.01 ± 0.01	4.35	74.14	0.04
Subset data	50				
Intercept		4.67 ± 0.81	---	---	---
Noise PC1		0.04 ± 0.06	0.07	45	0.80
Noise PC2		0.04 ± 0.10	1.15	45	0.29
Time of capture		-0.13 ± 0.07	3.58	45	0.06
Date of capture		-0.0004 ± 0.01	0.002	45	0.97
Activity center only	61, 57				
Intercept		5.78 ± 0.70	---	---	---
Noise PC1		0.18 ± 0.09	3.71	56	0.059
Noise PC2		-0.01 ± 0.07	0.02	56	0.90
Time of capture		-0.18 ± 0.07	4.36	56	0.04
Date of capture		-0.01 ± 0.01	7.48	56	0.01

[a] Number of observations, number of unique individuals.

Table 6.A7. Linear mixed effects and linear (subset data) models predicting body mass (g) from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	85, 80[a]				
Intercept		19.52 ± 0.59	---	---	---
Noise PC1		-0.06 ± 0.06	0.92	79.96	0.34
Noise PC2		-0.04 ± 0.09	0.26	75.43	0.61
Time of capture		0.07 ± 0.05	1.65	12.56	0.22
Date of capture		-0.002 ± 0.006	0.15	7.37	0.71
Subset data	49				
Intercept		19.77 ± 1.03	---	---	---
Noise PC1		-0.11 ± 0.07	1.79	44	0.19
Noise PC2		-0.25 ± 0.13	4.88	44	0.03
Time of capture		0.06 ± 0.09	0.53	44	0.47
Date of capture		-0.01 ± 0.01	0.32	44	0.57
Activity center only	59, 55				
Intercept		17.92 ± 0.88	---	---	---
Noise PC1		-0.03 ± 0.13	0.05	51.51	0.83
Noise PC2		0.03 ± 0.10	0.09	43.66	0.77
Time of capture		0.21 ± 0.08	7.07	26.06	0.01
Date of capture		0.001 ± 0.01	0.02	21.00	0.87

[a] Number of observations, number of unique individuals.

Table 6.A8. Linear mixed effects and linear (subset data) models predicting hematocrit (%) from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	88, 83[a]				
Intercept		50.10 ± 3.34	---	---	---
Noise PC1		-0.07 ± 0.29	0.06	83.00	0.80
Noise PC2		-0.05 ± 0.38	0.02	83.00	0.89
Time of capture		-0.07 ± 0.30	0.05	83.00	0.82
Date of capture		-0.01 ± 0.03	0.18	83.00	0.67
Subset data	50				
Intercept		56.82 ± 4.88	---	---	---
Noise PC1		-0.24 ± 0.34	1.41	44	0.24
Noise PC2		0.84 ± 0.61	4.68	44	0.04
Time of capture		-0.54 ± 0.41	1.76	44	0.19
Date of capture		-0.03 ± 0.06	0.21	44	0.65
Activity center only	62, 58				
Intercept		48.70 ± 4.05	---	---	---
Noise PC1		0.53 ± 0.54	0.94	51.51	0.83
Noise PC2		-0.08 ± 0.42	0.04	57.00	0.85
Time of capture		0.19 ± 0.37	0.26	57.00	0.61
Date of capture		-0.05 ± 0.04	1.51	57.00	0.22

[a] Number of observations, number of unique individuals.

Table 6.A9. Linear mixed effects and linear (subset data) models predicting feather PC1 (feather quality/condition at molt) from noise PC1 and noise PC2 in the entire dataset, in the subset data, and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	88, 83[a]				
Intercept		-0.01 ± 0.12	---	---	---
Noise PC1		-0.14 ± 0.08	3.20	79.90	0.08
Noise PC2		0.19 ± 0.11	3.16	75.32	0.08
Subset data	50				
Intercept		-0.33 ± 0.16	---	---	---
Noise PC1		-0.10 ± 0.09	1.36	45	0.25
Noise PC2		0.13 ± 0.15	0.74	45	0.39
Activity center only	62, 58				
Intercept		0.29 ± 0.22	---	---	---
Noise PC1		0.12 ± 0.16	0.57	55.90	0.45
Noise PC2		0.22 ± 0.13	2.81	52.12	0.09

[a] Number of observations, number of unique individuals.

Table 6.A10. Linear models predicting total antioxidant capacity (mM HOCl) from noise PC1 and noise PC2 in the entire dataset and within activity center males alone

	N	Estimate (β ± SE)	F	Denom (df)	P
Entire dataset	54				
Intercept[a]		5.50 ± 0.24	---	---	---
Noise PC1		0.01 ± 0.02	0.73	49	0.40
Noise PC2		0.01 ± 0.03	0.04	49	0.84
Time of capture		0.02 ± 0.02	0.63	49	0.43
Date of capture		-0.0001 ± 0.002	0.002	49	0.96
Activity center only	35				
Intercept		5.13 ± 0.37	---	---	---
Noise PC1		0.01 ± 0.04	0.09	30	0.77
Noise PC2		0.01 ± 0.03	0.02	30	0.90
Time of capture		0.05 ± 0.03	2.12	30	0.16
Date of capture		-0.001 ± 0.003	0.18	30	0.68

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