EFFECTS OF SOCIAL AGGRESSION ON TREE SWALLOW (*TACHYCINETA BICOLOR*) OFFSPRING BEHAVIOR AND PHYSIOLOGY

A Thesis
by
ALEXANDRA BEA BENTZ

Submitted to the Graduate School
Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2012
Department of Biology

EFFECTS OF SOCIAL AGGRESSION ON TREE SWALLOW (*TACHYCINETA BICOLOR*) OFFSPRING BEHAVIOR AND PHYSIOLOGY

A Thesis
by
ALEXANDRA BEA BENTZ
May 2012

APPROVED BY:

Lynn Siefferman
Chairperson, Thesis Committee

Robert Creed
Member, Thesis Committee

Susan Lappan
Member, Thesis Committee

Steven Seagle
Chairperson, Department of Biology

Edelma D. Huntley
Dean, Research and Graduate Studies

ABSTRACT

EFFECTS OF SOCIAL AGGRESSION ON TREE SWALLOW (*TACHYCINETA BICOLOR*) OFFSPRING BEHAVIOR AND PHYSIOLOGY. (May 2012)

Alexandra Bea Bentz, B.S., Appalachian State University

M.S., Appalachian State University

Chairperson: Lynn Siefferman

Territorial animals that breed in high-density environments often experience high levels of competition for resources necessary for reproduction and, thus, are likely to engage in social aggression with conspecifics. In many species, increased social aggression among breeding females results in increased concentrations of androgens (e.g., testosterone) in the females which are transferred to their offspring, a phenomenon called a maternal effect. The effects of increased yolk testosterone on nestling physiology have been well studied in birds. In general, elevated yolk testosterone increases nestling growth rates, but decreases immune function. Few studies have investigated the effect of yolk androgens on the development of behavioral syndromes. In these studies, nestlings exposed to elevated yolk testosterone tend to express more aggressive and bold behavioral traits. In this study, I altered the breeding density of tree swallows (*Tachycineta bicolor*) to modify levels of social aggression and yolk testosterone. I measured subsequent effects on nestling growth, overall health, and behaviors (aggression and boldness) using a partial cross-foster design. Females at high-density sites experienced more aggressive interactions and their eggs consequently had higher concentrations of testosterone. Nestlings hatched in high-density sites grew faster, were more aggressive, and bolder regardless of where they were reared. Final nestling mass was also negatively influenced by yolk corticosterone. Health was negatively affected by brood size. Nestling behaviors were not directly correlated; however, there was a positive trend in behaviors for nestlings reared and hatched at the same site. This study is one of the first to demonstrate that naturally-induced maternal effects adapt offspring phenotype to an anticipated environment.

ACKNOWLEDGEMENTS

This study was funded by grants from Sigma Xi, the American Ornithologists' Union, and the Biology Department. I would like to thank my committee chairperson, Dr. Lynn Siefferman, for always challenging me and pushing me to be successful, and for being a friend throughout the years. I would also like to thank my collaborator, Dr. Kristen Navara, for her contributions to the study and my committee members, Drs. Robert Creed and Susan Lappan, for their assistance in the development and execution of this thesis. I am grateful to Dave Hamilton and Maggie Hunter who provided invaluable help in the field and to the Valle Crucis Convention Center and private land owners for access to their land. Finally, I would like to thank my family and Edward Burress for their continual support and guidance.

TABLE OF CONTENTS

Abstract iv

Acknowledgements vi

List of Tables ix

List of Figures xi

Introduction 1

Methods 13

- Study Species 13
- Density Manipulation 14
- Habitat Suitability 15
- Partial Cross-Foster Design 16
- Parental Measurements 17
- Radioimmunoassay of yolk 18
- Nestling Physiology 19
- Nestling Behavior 20
- Statistical Analyses 22

Results 26

- Density Manipulation 26
- Nestling Physiology 27
- Nestling Behavior 28

Discussion 31

    Density Manipulation 31

    Nestling Physiology 33

    Nestling Behavior 36

    Conclusions 41

References 42

Tables and Figures 59

Biographical Information 75

LIST OF TABLES

Table 1. Independent *t*-test for differences in site parameters 60

Table 2. ANCOVA analyses of the effect of hatching site density and pair aggression on total number of interactions (vocal plus physical interactions; $F_{1,15} = 25.92$, $p < 0.001$) and type of interactions (vocal minus physical interactions; $F_{1,15} = 7.81$, $p = 0.004$). Interactions were observed during a 20 min period at each site for nests that were 100% complete on 5 different days over a span of 2 weeks. The interaction between site and aggression was removed from both models because $p > 0.4$.The mean (SE) number of interactions in low density (n=6) was 2.34 (0.70) and in high density (n=9) it was 4.66 (0.57). The mean (SE) interaction score (positive means more physical and negative means more vocal interactions) in low density (n=6) was 0.30 (0.42) and in high density (n=9) was -1.54 (0.34)..61

Table 3. ANCOVA to test predictors of yolk testosterone (ng/g). No interactions had $p > 0.1$ and were removed. The variables female age, clutch size, and first egg date were also removed (all $p > 0.6$). Overall model: $F_{2,16} = 3.17$, $p = 0.05$. The average yolk testosterone at low density sites was 1.34 +/- 0.26 ng/g and at high density it was 2.61 +/- 0.28 ng/g 61

Table 4. Mixed model analysis of effect of density on nestling H/L ratio. Variance (+/- SE) due to residuals is 0.01 +/- 0.002. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. Random effects were removed because they did not explain a significant proportion of the variance in the model; nest of origin ($Z = 1.16$, $p = 0.25$) or nest reared is ($Z = 0.06$, $p = 0.95$) 62

Table 5. Mixed models analysis for repeated measures testing effect of density on nestling growth. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to nest reared is 0.08 +/- 0.05 ($Z = 1.69$, $p = 0.09$), nest of origin is 0.17 +/- 0.09 ($Z = 1.80$, $p = 0.07$), and repeated measure is: age 2 = 0.04 +/-0.01, age 5 = 1.25 +/- 0.24, age 8 = 3.47 +/- 0.65, age 11 = 5.53 +/- 1.04, age 14 = 3.70 +/-0.72 (all $Z > 3.58$, $p < 0.001$). The mean growth (+/-SE) for nestlings hatched in low density is 13.06 +/- 0.17 g/d and hatched in high density is 13.43 +/- 0.18 g/d 63

Table 6. Mixed model analysis of effect of density on nestling breathing rate (controlled for by air temperature) during handling stress. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to residuals is 0.78 +/- 0.16. Random effects were removed because nest of origin explained no variance and nest reared (*Z* = 0.29, *p* = 0.77) was not significant. The mean aggression score (+/-SE) for nestlings hatched in low density is -0.30 +/- 0.19 and hatched in high density is 0.43 +/- 0.19..64

Table 7. Mixed model analysis of effect of density on nestling aggression score. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to nest reared is 0.40 +/- 0.21 (*Z* = 1.91, *p* = 0.057) and residuals is 0.53 +/- 0.12. Nest of origin was excluded because it was redundant and explained zero variance. The mean aggression score (+/-SE) for nestlings hatched in low density is -0.25 +/- 0.22 and hatched in high density is 0.30 +/-0.22 65

LIST OF FIGURES

Figure 1. Preliminary data from 2009; regression of average tree swallow breeding density and concentration of yolk testosterone ($r^2 = 0.56$, $F_{1,9} = 10.37$, $p = 0.012$)............66

Figure 2. Preliminary data from 2009; regression of yolk testosterone concentrations and average brood growth rate ($r^2 = 0.81$, $F_{1,7} = 26.20$, $p = 0.002$)...............................67

Figure 3. Positions in the nest occupied by nestlings when they received a feeding. Relative to nest entrance: position 1 was in the center of the nest, 2 was at the back of the nest, 3 was the left side, 4 was the right side, and 5 was below the entry hole 68

Figure 4. Yolk testosterone (ng/g) deposited in the 3rd egg of clutches from the high- and low-density sites. Error bars represent +/- 1 SE 68

Figure 5. Comparison of fostermates hatched in high- and low-density sites to detect differences in A) growth rate, B) breathing rate (controlled for by air temperature) during handling stress, and C) aggression during feeding. Siblings raised in the same nest are averaged and represented by circles; lines connect fostermates 69

Figure 6. Influence of brood size ($F_{1,59} = 9.60$, $p = 0.003$) on H/L ratio of nestling tree swallows...70

Figure 7. (A) Effect of yolk corticosterone ($F_{1,18} = 5.85$, $p = 0.03$) on growth prior to fledging in nestling tree swallows; below average yolk corticosterone is represented by dashed lines and bars and above average yolk corticosterone is represented by solid lines and bars. (B) Effect of hatch site density ($F_{1,186} = 5.28$, $p = 0.02$) on growth prior to fledging in nestling tree swallows; high-density hatch sites are represented by the solid line and bars and low-density hatch sites are represented by dashed line and bars. Bars represent +/- 1 SE..70

Figure 8. Relationship between begging rate and percent of feeds gotten by high aggression (closed circles and solid line; $r^2 = 0.16$, $F_{1,35} = 6.55$, $p = 0.02$) and low aggression (open circles; $r^2 = 0.03$, $F_{1,29} = 0.74$, $p = 0.40$) nestlings......................................71

Figure 9. Effect of site hatched on mean behavior scores: A) Breathing rate during handling stress (controlled for by air temperature; $F_{1,51} = 5.93$, $p = 0.018$) and B) aggression during feeding ($F_{1,43} = 4.26$, $p = 0.045$). Error bars represent +/- 1 SE..................72

Figure 10. Correlation of aggression and breathing rate score for nestlings that were A) swapped (reared in an environment they were not hatched; *r* = 0.02, df = 33, *p* = 0.93) and B) not swapped (raised in the environment in which they were hatched; *r* = 0.27, df = 34, *p* = 0.118)..73

Figure 11. Correlation for difference in feeding rate (biological parents feeding rate minus foster parents feeding rate) on A) breathing rate (*r* = 0.14, df = 31, *p* = 0.47) and B) aggression score (*r* = -0.31, df = 32, *p* = 0.08) that were reared in an environment they were not hatched 74

INTRODUCTION

Animals exhibit agonistic behavior toward conspecifics under many circumstances, including, but not limited to, the acquisition of mates, territory, and food, and in defense of offspring. Aggression in females of territorial and monogamous songbirds can be beneficial, because more-aggressive females are better able to acquire or maintain breeding territories, to pair with a monogamous mate, and are less likely to experience brood parasitism (Moller 1987; Gowaty and Wagner 1988; Dunn and Hannon 1991; Rosvall 2008). Several correlative studies show a positive relationship between aggression among breeding females and concentrations of androgens, e.g. testosterone, in their egg yolks (Whittingham and Schwabl 2001; Mazuc et al. 2003; Hargitai et al. 2009). By experimentally inducing aggression in wild eastern bluebirds (*Sialia sialis*) during the period of egg yolking, Navara et al. (2006b) showed that concentrations of yolk testosterone increased. Environmentally-induced variation in egg hormones is a classic example of a maternal effect; a non-genetic mechanism in which the conditions females experience affect offspring phenotype (Groothuis et al. 2005; Wolf and Wade 2009).

The physiological mechanism responsible for the maternal transfer of androgens to offspring occurs within the ovarian follicles that surround the developing oocyte and yolk. Each ovarian follicle has three steroidogenic layers in the follicle wall; the innermost granulosa cells produce progestins (P_4), the theca interna cells produce androstenedione (A_4), testosterone (T), and dihydrotestosterone (DHT), and the outermost theca externa cells produce estrogens (E_2; Groothuis and Schwabl 2008). The activity of these cells dictates the transfer of hormones from mother to offspring and is regulated by the stage of follicle development and concentrations of luteinizing (LH) and follicle-stimulating hormones (FSH) from the pituitary (Gil 2008; Groothuis and Schwabl 2008). LH and FSH are consequently regulated by gonadotropin-releasing hormone from the central nervous system in response to internal and external stimuli, such as social aggression (Gil 2008; Groothuis and Schwabl 2008). The transfer of hormones from female to egg is also assisted by the lipophilic nature of androgen hormones and yolk, which causes the preferential movement of androgens from the female to the egg (Groothuis et al. 2005; Carere and Balthazart 2007). In fact, the highest levels of androgens are produced during the period of rapid yolk deposition (Marrone and Hertelendy 1983). To date, it is unclear whether females have control over this mechanism of hormone transfer (Groothuis and Schwabl 2008).

Three alternative mechanisms for maternal transfer of androgens have been proposed. The Physiological Epiphenomenon Hypothesis suggests that the concentration of yolk hormones is a reflection of the concentration of these hormones in the female (Groothuis and Schwabl 2008). If this hypothesis is correct, the concentration of hormones in the egg and the female should exhibit a positive relationship (Schwabl 1996a; Jawor et al. 2007). This hypothesis accounts for the preferential movement of yolk androgens from the female to the egg due to the lipophilic nature of androgens and yolk and is, therefore, the most parsimonious hypothesis (Groothuis and Schwabl 2008). The Flexible Distribution Hypothesis proposes that the concentration of hormones in the egg and the female are regulated based on the optimal hormone levels for each individual (Groothuis and Schwabl 2008). Based on this hypothesis, the egg has the potential to sequester hormones when above-optimal levels are circulating in the mother (Navara et al. 2006b; Groothuis and

Schwabl 2008). This could be beneficial because increased androgen levels in females have been shown to interrupt ovulation and other processes associated with reproduction (Searcy 1988). This can also account for negative correlations between female and egg hormone concentrations (Mazuc et al. 2003; Verbovan et al. 2003; Navara et al. 2006b). The Independent Regulation Hypothesis suggests that hormones in the egg and the female are regulated independently (Groothuis and Schwabl 2008). This offers an explanation for data that demonstrate no correlation between mother and egg hormone concentrations (Groothuis et al. 2005). Support for this hypothesis lies in a study performed with a lizard species (Lovern et al. 2003), but examples from avian systems are currently lacking. This is the least probable hypothesis because it requires that the mechanism responsible for regulating hormone production and distribution in eggs is independent of the female, which is unlikely (Groothuis and Schwabl 2008).

Although the mechanism of hormone transfer may not be agreed upon, data clearly show that factors such as the environment and female condition influence how females distribute yolk androgens. For example, high breeding density often increases levels of social aggression; thus, the relationship between density and yolk T is expected. Indeed, in European starlings (*Sturnus vulgaris*; Pilz and Smith 2004), tree swallows (*Tachycineta bicolor*; Whittingham and Scwabl 2001), house sparrows (*Passer domesticus*; Mazuc et al. 2003), American coots (*Fulica americana*; Reed and Vleck 2001), and collard flycatchers (*Ficedula albicollis*; Hargitai et al. 2009) females that nest at higher densities lay eggs with significantly higher T levels and researchers generally attribute this to increased female aggression in high-density environments. As evidence of this, Male et al. (2006) showed that tree swallows nesting in higher densities are involved in more aggressive interactions.

Researchers have suggested that this variation in hormone deposition likely serves an adaptive purpose. Maternal effects could be an adaptive means by which a female can change the phenotype of her offspring hormonally in preparation for a given environment (Mousseau and Fox 1998; Groothuis et al. 2005; Carere and Balthazart 2007). Increased yolk androgens have been shown to increase offspring growth, which could be an advantage for offspring fledging into a competitive, high-density environment (Pilz et al. 2004; Muller et al. 2007).

The physiological effects of increased yolk androgens on offspring phenotype are numerous and varying, but enhanced growth is thought to be one of the most beneficial (Pilz et al. 2004; Muller et al. 2007). One of the first studies injected T into canary (*Serinus canaria*) egg yolks and demonstrated that nestlings grew larger and begged more often compared to controls (Schwabl 1996b). In red-wing blackbird (*Agelaius phoeniceus*) nestlings, yolk T injections cause greater development of neck muscles, which facilitates vigorous begging (Lipar and Ketterson 2000). The increase in muscle mass should be favorable, because nestling red-wing blackbirds that extend their necks the farthest while begging are fed more often by parents (Teather 1992). In eastern bluebirds (Navara et al. 2005) and house finches (*Carpodacus mexicanus;* Navara et al. 2006a) yolk T is positively related to offspring growth. Other studies have found sex-specific effects of yolk T on offspring size. Male collared flycatcher nestlings hatching from T-injected eggs experience reduced growth, while female nestlings experience enhanced growth (Pitala et al. 2009). A similar phenomenon occurs in zebra finches (*Taeniopygia guttata*) resulting from T injections of both mothers (Rutkowska et al. 2007) and eggs (von Engelhardt et al. 2006). Still other studies demonstrate either no effect or a negative effect of yolk T on offspring growth (Andersson et al. 2004; Rubolini et al. 2006). Interestingly, studies of Passeriformes (songbirds) consistently demonstrate positive results (Schwabl 1996b; Navara et al. 2005; Navara et al. 2006a) while studies of Galliformes (game birds) and Charadriiformes (shorebirds) tend to fail to demonstrate a positive relationship (Andersson et al. 2004; Rubolini et al. 2006).

The majority of studies involving the effect of T on nestling quality indicate a tradeoff; enhanced nestling growth is thought to come at the cost of the immune system (Groothuis et al.2005). One way in which T can compromise the immune system is by inducing oxidative stress, which is responsible for nonspecific tissue damage (von Schantz et al. 1999). The Immunocompetence-Handicap Hypothesis was one of the first to suggest that a trade-off could exist between the beneficial effects of hormones and immunosuppression (Folstad and Karter 1992). Braude et al. (1999) challenged this hypothesis by proposing that rather than immunosuppression, immunoredistribution, or a temporary shifting of leukocytes to damaged tissues, occurs. These two hypotheses were reconciled by Kurtz et al. (2000), who proposed that both could be occurring. Immunosuppression prevents the autoimmune response that could arise in reaction to the tissue damage associated with oxidative stress while immunoredistribution directs cells to places of tissue damage. A trade-off, however, is still expected to occur because immunity is costly and only those individuals that are in superior condition should be able to withstand a lowered immune defense during redistribution (Kurtz et al. 2000).

A weakened immune system is one of the most-researched detrimental effects of increased T exposure and also one of the most variable. In adults, T manipulations have shown that increased T concentrations in plasma result in a decreased humoral immune response (Peters 2000; Alonso-Alvarez et al. 2007), but free-living males with naturally higher T levels exhibit higher humoral immune responses (Peters 2000). In some species, nestlings hatching from T-injected eggs demonstrate a compromised cellular immune response (eastern bluebirds; Navara et al. 2005), and, in other species, a superior cellular immune response (house finches; Navara et al. 2006a). Again, the effect of egg T may be sex specific; after injecting mothers with T during egg laying Rutkowska et al. (2007) found that sons exhibited impaired cellular immune responses while those of daughters were enhanced. Other studies have found no effect of egg T injections on nestling immunocompetence (Rubolini et al. 2006; Pitala et al. 2009). Other detrimental effects of increased yolk T on nestlings include decreased hatching success (Navara et al. 2005) and increased resting metabolic rates (Tobler et al. 2007).

Maternal hormones transferred to developing offspring tend to exert their effect either before or shortly after hatching. Few studies, however, have attempted to document whether the physiological effects of maternally-derived hormones persist into adulthood. Rutkowska et al. (2007) found an effect of yolk androgens on nestling zebra finch growth and immune function, but found no long-term physiological effects on fecundity or attractiveness. This could be because androgens act early in development, during a period in which irreversible changes are made to offspring. During embryonic stages, androgen receptors are expressed in the hindbrain, hypoglossal motor nucleus, supraspinal motor nucleus, and the syrinx (Groothuis and Schwabl 2008). Consequently, early androgen exposure can cause androgen receptors to be up-regulated leading to an increased sensitivity to these hormones. Early exposure can also alter androgen production and action of several endocrine glands such as the hypothalamus, pituitary, gonadal, and adrenal gland (Dufty et al. 2002; Carere and Balthazart 2007; Groothuis and Schwabl 2008). Therefore, there is a much greater potential for early androgen exposure to affect long-term behaviors rather than physiology. Early androgen exposure can also cause enduring changes to behavior through juvenile social experiences shaped by the effects of yolk androgens (Kassel and Davis 1975; Groothuis 1992). Eising et al. (2006) found that the behavioral effects of injected yolk T persisted one year after black-headed gulls (*Larus ridibundus*) hatched. Other studies found that juveniles hatched from T-injected eggs are more aggressive (Strasser and Schwabl 2004) and more explorative (Tobler and Sandell 2007; Ruuskanen and Laaksonen 2010) at sexual maturity.

Despite the consensus in the literature that androgens facilitate behavioral adjustments in individuals (Oliveira 2004), the effects of yolk androgens on offspring physiology have received much more attention than the effects on offspring behavior. This is particularly noteworthy because personality can have strong effects on fitness (Sih 2011). Differences in personality (aka “behavioral syndromes”) have been documented in wild animals and are characterized as suites of correlated behaviors that are consistent across contexts (Gosling and John 1999; Koolhaas et al. 1999; Sih et al. 2004b; Duckworth 2010). In one of the earliest behavioral studies, Huntingford (1976) found that aggression toward conspecific intruders and boldness in the presence of predators were positively related. In general, aggression and boldness are the two behavioral assays that are most commonly measured and often correlate positively. The combination of these two assays has led to two distinct behavioral syndromes: proactive and reactive. Proactive individuals are characterized as being more aggressive, bolder, and having higher levels of T as opposed to reactive individuals (Hessing et al. 1994; Koolhaas et al. 1999). Currently researchers do not agree as to whether these behaviors correlate because of similar selection pressures or a physiological constraint, such as being controlled by the same gene (Moretz et al. 2007). Certainly behaviors have been shown to have a genetic component (Drent et al. 2003; Fidler et al. 2007); however, the genetic contribution should not suggest that behaviors are inflexible, but are instead a foundation for early environmental contributions to create adaptive phenotypes. Recent maternal effects studies suggest that animals have limited behavioral plasticity which operates within the confines of their genotype in combination with early environmental pressures (Eising and Groothuis 2003; Daisley et al. 2005; Eising et al. 2006; von Engelhardt et al. 2006; Tobler and Sandell 2007; Muller et al. 2009).

The best-documented behavioral response to increased yolk androgens are competitive interactions among begging siblings. Nestlings from eggs injected with T tend to beg more and obtain more food, possibly leading to enhanced growth (Eising and Groothuis 2003; von Engelhardt et al. 2006). Experimentally enhanced yolk androgen concentrations in eggs have also led to more-exaggerated territorial and aggressive behaviors in nestlings (Eising et al. 2006; Muller et al. 2009) as well as more explorative behavior when confronted with a novel object (Tobler and Sandell 2007). Daisley et al. (2005) found that Japanese quail (*Coturnix japonica*) nestlings from eggs injected with T were more likely to have a bolder behavioral strategy and approach novel objects much faster. Sex-specific effects have also been found. Male pied flycatchers (*Ficedula hypoleuca*) from T-injected eggs were more explorative at maturity than control males, while females showed no difference (Ruuskanen and Laaksonen 2010). Unfortunately, few studies have measured multiple behavioral traits in response to increased yolk androgens. Ruuskanen and Laaksonen (2010) measured both explorative and aggressive behaviors in relation to yolk T injections, but found that they were not correlated; suggesting yolk androgens can influence behavioral traits, but not behavioral syndromes in the absence of a selection pressure.

Pre-natal hormonal provisioning causes significant changes to nestling physiology and behavior, and while maternal effects influence the fitness of both females and their offspring, selection typically maximizes maternal rather than offspring fitness. Therefore, the adaptive role of maternal effects is considered in a framework concentrating on maternal fitness. Four different adaptive scenarios have been identified: anticipatory, selfish, bet-hedging, and transmissive maternal effects. Anticipatory maternal effects increase the maternal fitness by increasing offspring fitness; females adjust the phenotype of their offspring according to environmental conditions to maximize offspring fitness (Marshall and Uller 2007). This typically occurs when environments are predictable. For example, females in a high-density environment that transfer more yolk T, will create more competitive offspring and increase their survival post-fledging, resulting in positive selection of this hormonal response (Marshall and Uller 2007). Selfish maternal effects occur when females increase their own fitness at the expense of their offspring's fitness. This happens most often when mothers reproduce multiple times, causing them to decrease current reproductive efforts in favor of future attempts (Marshall and Uller 2007). Bet-hedging maternal effects occur if the environment is not predictable, causing females to vary yolk androgens to produce a range of offspring phenotypes in hopes that one is favorable (Marshall and Uller 2007). Transmissive maternal effects reduce both maternal and offspring fitness and are maladaptive (Marshall and Uller 2007). These occur when physiological constraints prevent the buffering of maternal hormones being transmitted despite their being selected against. For example, if females in high-density habitats are in poor condition or suffer from high predation, they will be stressed and therefore transfer more stress hormones, e.g. glucocorticoids, to their egg yolk (Love et al. 2008, Saino et al. 2005, Marshall and Uller

2007). Nestlings from eggs with high concentrations of corticosterone, a predominant glucocorticoid, tend to be of lower quality, they grow more slowly, have more reactive personalities, beg less, and have a depressed immune system (Hayward and Wingfield 2004; Rubolini et al. 2005). Furthermore, corticosterone levels have been shown to depress T levels (Okuliarova et al. 2010, Henriksen et al. 2011). Therefore, high yolk corticosterone is detrimental in high-density environments.

In determining the adaptive influence of maternal effects it is important to examine the context in which studies are executed. Most studies have investigated the effects of maternal hormone transfer on offspring by experimentally increasing yolk androgens. There are many negative effects associated with hormone injections aside from the obvious physical harm it can exert on the egg (Groothuis and von Engelhardt 2005; Navara et al. 2005). It can be challenging to administer physiologically-relevant doses of steroids because they are often species specific and, in many species, the endogenous hormone levels, or those transferred from the mother, are unknown (Elf and Fivizzani 2002; Groothuis et al. 2005; Groothuis and von Engelhardt 2005; Carere and Balthazart 2007). Hormone injections may also cause hormone levels to exceed that with which an individual can naturally cope (Kurtz et al. 2000; Groothuis and von Engelhardt 2005) and, therefore, experimental manipulations of androgens can provide misleading, maladaptive results. For example, Peters (2000) found that the physiological effect of T varied between manipulated and free-living males; males with experimentally increased T had a decreased immune response while males with naturally higher T had a higher immune response, suggesting Peters exceeded what individuals could naturally cope when using injections. The number and type of hormones injected is also of concern. Most studies inject one androgen; however, T, DHT, A_4, and E_2

are all found in high concentrations in eggs and the injection of just one may exclude all possible hormonal interactions contributing to phenotypic change (Carere and Balthazart 2007).

Another factor that few studies have taken into consideration is that the pre-natal environment is influenced by maternal hormones, while the post-natal environment is influenced by parental and environmental factors, yet both are expected to influence offspring phenotype. To separate pre-natal and post-natal effects, a cross-foster design is necessary (Bernardo 1996). Only a few studies investigating the effect of maternal hormones on offspring phenotype have performed a cross-foster experiment to separate the pre-laying and post-laying maternal environment (Eising et al. 2006; Rutkowska et al. 2007; Tobler et al. 2007).

In this study, I experimentally manipulated breeding density of tree swallows and measured subsequent yolk T and corticosterone concentrations, and nestling growth, health, and behavior (aggression and boldness). Yolk T is expected to be positively correlated with density because of increased agonistic interactions (Whittingham and Schwabl 2001); while corticosterone is expected to be negatively correlated with density due to increased predator vigilance in high-density environments (Love et al. 2008). By measuring the aggression and boldness of parents and then incorporating a cross-foster design, I tested the relative importance of pre-natal breeding density versus parental personality and post-natal environment in influencing offspring phenotype. If personality is influenced by yolk androgens, then those offspring with greater amounts of yolk T should display increased growth, decreased health, and increased proactive behaviors regardless of parental behaviors or post-natal environment. If behaviors are strongly heritable (influenced by parental behaviors and not yolk androgens), then offspring behaviors should closely mimic those of their parents regardless of the density at the site at which they were hatched. Furthermore, if yolk androgens have no influence on offspring phenotype, but rearing environment does, then foster-mates should show similar phenotypes regardless of their hatching environment. This study is one of the first to determine if a naturally-induced maternal effect can act as a proximate mechanism influencing the development of offspring phenotype in anticipation of a particular environment. If higher yolk T in eggs laid at higher-density sites causes nestling phenotypes to be characterized by faster growth and more proactive behaviors, then maternal effects are likely an adaptive mechanism that can prepare offspring for competitive environments.

METHODS

Study Species

While hormone-mediated maternal effects are universal in the animal kingdom, birds are model species. Viviparous organisms can alter hormone concentrations throughout gestation, making it difficult to quantify maternal hormone provisioning. However, in oviparous organisms, such as birds, there is a discrete period of hormonal provisioning during follicle maturation and the female can no longer influence hormone concentrations after ovulation occurs and the egg is released. If eggs are collected and analyzed in the early embryonic stages, prior to endogenous hormone production, maternal hormone investment can easily be quantified (Groothuis et al. 2005). Furthermore, birds transfer substantial maternal hormones to the yolk (Schwabl 1993, Groothuis et al. 2005) and bird behavior is well-studied (Groothuis et al. 2005).

For this research, I studied tree swallows, a socially-monogamous passerine, in Watauga County, North Carolina (36^{o}12'41"N, 81^{o}40'7"W) between April and July in 2011. Tree swallows are one of the most-studied birds in North American, in part, because they are secondary-cavity nesters that readily accept nest boxes. They typically lay one brood per season and prefer to nest in open fields and marshes where they forage aerially for insects (Robertson et al. 1992). Female tree swallows are particularly appropriate for T-related research, because they aggressively compete for their cavities (Rosvall 2008). This species breeds in a variety of nesting densities and nesting density is positively related to the number of agonistic interactions (Male et al. 2006). Also, female tree swallows do not vary yolk androgen concentrations within a clutch, but rather between clutches in response to environmental factors such as breeding density (Whittingham and Schwabl 2001). Indeed, in a preliminary study at my field site during 2009, I documented a positive relationship between breeding density and yolk T (using the 3rd egg of each clutch; fig. 1) and between yolk T and brood average growth (fig. 2).

Density Manipulation

I manipulated breeding density creating high- and low-density sites; thereby, altering aggression among female tree swallows. Breeding densities were measured by delineating a 300 m radius around each nest box in ArcGIS v. 10 (ESRI, Redlands, CA) and recording the proportion of active nests during each pair's breeding cycle. Tree swallows typically remain within 100-300 m of the breeding site when foraging, so a 300 m radius should encompass the area within which a female could potentially come into contact with conspecifics (McCarty and Winkler 1999). In the high-density site, 53 nest boxes were placed so that no box was >12 m from the nearest nest box, because tree swallows have been shown to defend a territory around their nest box of 6-8 m (Robertson and Gibbs 1982). All nest boxes (53) fit within each individual box's 300 m radius when measuring breeding density, allowing each box at a site to have the same potential density. In the five low-density sites, 50 nest boxes were placed so that no box was <36 m apart, because Muldal et al. (1985) showed that tree swallows prefer to nest as far apart as possible within a 36 m radius. Again, nest boxes (~11) fit within the 300 m radius of each individual's nest box.

Although Male et al. (2006) found that the number of social interactions increased in high-density areas, I observed randomly selected pairs for vocal and physical interactions for

20 min once nest building was complete to verify that the experiment influenced social interactions. Observations were done on five different days over a span of 2 weeks. To determine total number of interactions, all physical and vocal interactions were combined. To determine prevalence of interaction type, the number of vocal interactions was subtracted from number of physical interactions; therefore, pairs that engage in equal types of interactions have a score of zero, those that engage in more physical interactions have a positive score, and those that engage in more vocal interactions have a negative score.

Habitat Suitability

Due to the nature of the experimental design, the field sites could not be placed in the same field. The high-density site was in one field and the low-density sites were split between 5 sites. All nest boxes were placed in open fields and field sites were no more than 3 mi apart. To account for differences in habitat quality that could influence settlement patterns or individuals' condition, I used ArcGIS v. 10 (ESRI, Redlands, CA) to determine habitat suitability. Maps of Watauga County were obtained from the ArcSDE database maintained by Appalachian State University's Geography and Planning Department. Distance to water, forest edge, and human structures were considered to be variables that influence habitat suitability, because they affect food availability and interspecific competition. Tree swallows are aerial insectivores that rely on insects emerging from aquatic environments, hence, proximity to water is desirable (Robertson et al. 1992). House wrens (*Troglodytes aedon*) typically nest near the forest edge and are fierce competitors for nest cavities; usually defending multiple nests which they obtain by destroying the nests of other species (Finch 1990; Rendell and Robertson 1989; Robertson et al. 1992). As a result, nest boxes farther from the forest edge are best and I have found that tree swallow density is greatest farther from the forest (Bentz and Siefferman, unpubl.). Purple martins (*Progne subis*) and barn swallows (*Hirundo rustica*) are also interspecific competitors for aerial insects and cavities; however, these competitors rely on human structures (Brown 1997; Brown and Brown 1999). Houses sparrows (*Passer domesticus*) are also competitors for nesting cavities, but they also prefer to nest near human activity (Munro and Rounds 1985). Therefore, being farther from human structures is better. Using the aforementioned variables, I calculated the straight-line distance to water, forest edge, and human structures for each nest box. Distances were reclassified on a 20 pt scale; a score of 20 indicates that the box was close to water, far from human structures, and far from the forest edge. The reclassified variables were then put in the following weighted equation: (0.10*human structure) + (0.45*water) + (0.45*forest edge). The distance to human structures was weighted less because the species attracted to human structures are not commonly found at my field sites. This generated a comprehensive scale from 1-20 for each nest box ranking its habitat suitability (higher scores being more desirable).

Partial Cross-Foster Design

To further account for differences at high- and low-density sites, I performed a partial cross-foster experiment. I exchanged nestlings of comparable size between broods that were hatched on the same day at different densities on day 2 post-hatch (day 1 = hatch date). Half of the nestlings from a low-density site brood were swapped with half of the nestlings from a high-density site brood. This occurred at 20 nests (10 at each site density). In this way, I was able to compare the phenotype of nestlings reared in the same environmental conditions, but that were hatched in either high- or low-density environments. This design allowed me to separate the effects of pre-natal hormones and post-natal environment on nestling phenotype.

Parental Measurements

I assessed aggression level of each breeding pair during the incubation stage by presenting them with a conspecific model and a playback of tree swallow chatter at their nest box for 5 min and counting the number of times the pair aggressively flew by, hovered, or attacked the model. An aggression score from 1-6 was calculated based on Duckworth (2010). Parental feeding rates were measured during the aggression trials for nestlings (see methods below) as number of feeding visits per minute. Parental boldness was measured as the number of feeding opportunities missed following a “predator” intrusion (my appearance at the box to initiate the camera for the nestling aggression trials). On average it took pairs (mean ± SE) 11.6 ± 2.0 min to resume feeding with a range of 7.0 – 40.9 min. The number of minutes following my intrusion before they resumed feeding was divided by their feeding rate to determine how many feeds they missed. Bolder individuals should return more quickly to feed nestlings following an intrusion. I also captured each female and measured mass (± 0.1 g) and wing chord (± 0.1 mm). I created an index of female body condition using residuals of a regression of mass on wing length ($r^2 = 0.11$, $F_{1,51} = 6.28$, $p = 0.016$; Brown 1996). Therefore, a positive body condition indicates that they are heavier for their wing size and this may indicate these birds experience decreased flight ability (Rosvall 2011). I estimated female age as second year (SY) or after-second year (ASY) using plumage coloration (Hussell 1983).

Once eggs were laid, I recorded clutch size and measured the average egg mass to the nearest 0.01 g (total mass of clutch divided by clutch size). To determine if females at the high-density site transfer more T to their eggs, I collected the 3rd egg from each clutch prior to incubation and endogenous hormone production, so that hormones in the egg would be maternally-derived (Elf and Fivizzani 2002). Eggs were then frozen at -20° C so that yolk T and corticosterone could be measured via radioimmunoassay (see detailed methods below).

Radioimmunoassay of yolk

Yolk hormones, T and corticosterone, were extracted from homogenized yolk samples with a double ether extraction followed by liquid column chromatography according to methods described by Schwabl (1993). Briefly, 15 mg of yolk was weighed and vortexed with 1000 µl of deionized water. The percentage of mass the yolk comprised in each egg was calculated as % yolk. Percent yolk is a measure of egg quality, because yolk contains lipids and immunoglobulins (Hartmann and Wilhelmson 2003); furthermore, Ricklefs (1984) showed that relative size of the yolk was a major component in explaining egg composition. Next, 3 ml of petroleum:diethyl ether (30:70 vol/vol) was added, the mixture was vortexed for 30 s and was allowed to settle for 20 min. Samples were then snap frozen and the supernatant was poured off and dried using a N_2 stream. The sample was reconstituted in 1.0 ml 10% ethyl acetate in isooctane and individual steroids were separated using celite column chromatography. Steroids were eluted in the following fractions: T - 10% and corticosterone - 50% ethyl acetate in isooctane. T and corticosterone were quantified using standard competitive binding radioimmunoassays (using Anti-T and Anti-corticosterone from MP Biomedicals, Solon, OH) as described in Wingfield and Farner (1975). All samples were done in one assay for each hormone. Average recoveries were 89.3% for T and 85.5% for corticosterone. Intrassay variation was 2.97% for T and 1.93% for corticosterone.

Nestling Physiology

Blood samples (~120 µl) were taken from nestlings at age 14 days post-hatch by veinpuncture of the cutaneous ulnar vein. A blood smear was made from the samples and stained with Wright-Giemsa stain (Sigma-Aldrich, St. Louis, MO, USA, WG128). The heterophil/lymphocyte (H/L) ratio was estimated from the number of heterophils and lymphocytes per 100 leukocytes (Dein 1986). The H/L ratio is a good measure of reaction to stressors including exposure to new social interactions and environmental variables (Gross and Siegel 1983; Brown 1996; Vleck et al. 2000; Muller et al. 2011). Stressors cause an increase in heterophils and a decrease in lymphocytes leading to higher H/L ratios. Rather than being a transient hormonal reaction such as that observed with stress hormones, H/L ratios reflect stressors over a longer period, because leukocyte numbers respond more slowly than hormones (Brown 1996; Vleck et al. 2000). H/L ratios are also thought to reflect overall immunity (Moller and Petrie 2002; Owen and Moore 2008). For example, birds suffering from bacterial infection have higher H/L ratios (Davis et al. 2004) and poor energetic condition can reduce lymphocyte counts (Owen and Moore 2008).

I assessed nestling growth by measuring mass (± 0.1 g) and wing chord (± 0.1 mm) on days 2, 5, 8, 11, and 14 post-hatch. Growth rates were derived from the slope of a linear regression of chick mass on age 2-11 days (for all nestlings: $0.96 < r^2 < 0.99$; Hinde et al. 2009). I calculated indices of body condition of day 14 nestlings using residuals from a linear regression of mass on wing size ($r^2 = 0.19$, $F_{1,107} = 25.45$, $p < 0.001$; Brown 1996). Nestlings were banded on day 8 post-hatch with USFWS bands.

Nestling Behavior

I measured nestling boldness at age 14 days (at least 2 hr apart from the aggression trial) using average breathing rate (sympathetic response) during handling stress as a proxy for boldness. Fucikova et al. (2009) showed that breathing rate during handling stress in 14 day old great tits was repeatable 6 mo later and was positively correlated with boldness (using a novel environment test). Bold individuals tend to have more reactive sympathetic responses and respond to stressors with a higher breathing rate than shy individuals (Koolhaas et al. 1999). Each nestling was measured twice and repeatability was calculated using the intraclass correlation coefficient (Lessells and Boag 1987). Nestling mass was not related to breathing rate ($r^2 = 0.03$, $F_{1,65} = 1.76$, $p = 0.19$); however, because warmer air temperatures can cause an increase in respiration in birds I tested effects of temperature (Salt 1964). Air temperature significantly influenced breathing rate ($r^2 = 0.08$, $F_{1,67} = 6.08$, $p = 0.016$); therefore, breathing rate scores are residuals of a regression of breathing rate on air temperature so that higher scores mean a higher breathing rate for a given air temperature.

I measured nestling aggression using food competition trials conducted at age 14 days post-hatch (+/- 1 day). Due to constraints placed on the parents by the design of the nest boxes, parents preferentially feed offspring nearest the entrance to the box, thus, competition among offspring for this location is expected (McRae et al. 1993). I mounted small cameras adjusted for low light (HRC-20HEX; SCS, Inc., Montebello, NY, USA) within the nest boxes. I blocked the nest box for 1 hr to ensure the nestlings were hungry and to allow parents to habituate to the presence of the camera. After this time, I re-opened the nest box and allowed the parents to visit the nest box twice to ensure that parents had habituated to the cameras. I then removed nestlings from the nest, began the recording, and returned nestlings in order of band number. Because my presence caused the parents to perceive me as a threat (they responded aggressively), I measured their latency to resume feeding as ‘parental boldness.’ Upon starting the camera, I recorded parental provisioning for 1 hr. While transcribing the video footage, I sequentially labeled nestlings as they were replaced in the nest. This allowed me to follow each nestling and transcribe its behavior in the nest. This also allowed an unbiased viewing, because during the time that I transcribed video footage, I was unaware of nestling band numbers (and their hatching site). It is unlikely that the order in which I positioned nestlings in the nest influenced begging position, because nestlings tended to move location immediately after I placed them in the box. I quantified aggressive interactions of offspring based on their begging posture (1 = gaping, 2 = gaping, facing up, 3 = gaping, extending neck, and 4 = gaping, lifting body; Pilz et al. 2004) and location in the nest while begging (relative to nest entrance: 1 = center, 2 = back, 3 = left side, 4 = right side, 5 = nearest hole; McRae et al. 1993). Aggression score is a principal component of average posture and % of time in ideal feeding spot (Eigenvalue = 1.332, 66.63%; loading values: Average Posture = 0.816, % time in ideal spot = 0.816). I used a Chi-square test to determine the ideal position in the nest. I found two locations in the nest where nestlings were fed more often than expected; positions 1 (center) and 5 (nearest the hole) ($\chi^2 = 38.92$, df = 4, $p < 0.001$; fig. 3). Therefore, nestlings with a higher aggression score begged more vigorously and occupied ideal feeding spots for a greater percentage of their begging bouts.

Nestlings with either positive or negative scores for both behavioral traits were given a personality ranking. Nestlings were given a personality rank as reactive (N = 15) if they had both negative scores in aggression and breathing rate, and a rank as proactive (N = 22) if they had both positive scores in aggression and breathing rate; individuals with a mixture of positive and negative scores (N = 30) were not given a specific personality rank.

Statistical Analyses

All data were tested for normality with a Shapiro-Wilk test (all $p > 0.06$), only yolk corticosterone was non-normal and was transformed with Log10 to approximate normality. SPSS (ver. 17.0; SPSS, Inc., Chicago, IL) was used to analyze data; all tests are two-tailed with a significance level set at $p \leq 0.05$. Unless otherwise mentioned, all means are followed by standard error. A Chi-square analysis was used to determine if high- versus low-density breeding sites were more likely to be used by SY versus ASY females and t-tests were used to compare reproductive performance and condition of birds at high- and low-density sites. I used an ANCOVA to determine whether aggressive interactions among adults, yolk T, or yolk corticosterone were influenced by site density. The covariates used were pair aggression for the social aggression model and female body condition, aggression, and habitat suitability for the hormone models. No interactions were significant ($p < 0.1$) and all were removed from the models.

Initially, I used paired *t*-tests to determine whether nestling hatch site influenced physiology (growth rates and H/L ratio) and behavior (aggression and boldness). Scores of siblings raised within the same nest were averaged and they were paired with fostermates.

I used Linear Mixed Models to determine whether nestling physiology (H/L ratio) or behavior (aggression and boldness) was influenced by site density or other confounding variables. A Repeated Measures Mixed Model was used to test nestling growth using age (days 2, 5, 8, 11, and 14 post-hatch) as the repeated measure and nestling ID as the subject. Using mixed models allowed me to include all variation in nestling phenotype rather than brood averages. The fixed effects I tested in each model included: density manipulation (hatch site and reared site: 0 = low density, 1 = high density), female age (0 = SY or 1= ASY), and swap status (0 = raised in a nest other than the one hatched, 1 = raised in same nest as hatched). Additionally, I included nestling age as a fixed factor in the repeated measures model. Female age ($\chi^2 < 2.60$, $p > 0.11$) and swap status ($\chi^2 < 0.85$, $p > 0.36$) were not significant in any models and were not included in subsequent analyses. To further verify that parents did not favor biological over foster offspring, I found that biological nestlings did not receive a greater percent of feeds compared to foster nestlings ($t_{2,68} = -0.63$, $p = 0.53$).

The covariates in each model included hatch date, brood size, habitat suitability, nestling body condition at fledging, % yolk, yolk testosterone, and yolk corticosterone. Additionally, I included parental aggression scores and feeding rate as covariates when testing nestling aggression; parental boldness as a covariate when testing nestling boldness; and feeding rate, begging rate (number of times nestling begged per feeding bout), and begging success (times fed divided by times begged) when testing nestling growth. Furthermore, I performed a linear regression analysis to determine if aggression and begging rate influenced the number of feeds nestlings received. For this analysis, aggression scores were categorized as high aggression (positive scores) and low aggression (negative scores).

In all mixed models, I first tested for multicollinearity among the predictor variables with a regression model and used collinearity diagnostics; all VIF < 2. I also performed a correlational matrix and for all variables $r < 0.7$. All models use absolute parameter, likelihood, hessian, and convergence settings. Random effects included were the nest of origin and nest reared; however, if a random effect was not significant (tested with Wald *Z*) or did not improve the model fit it was excluded. In all cases, removal of random effects did not alter the significance of main effects.

I fitted each mixed model using a -2 restricted likelihood ratio test (LRT) to determine if random and main effects significantly improved the model fit and should thus be incorporated. The procedure included calculating the difference in the -2 restricted log likelihood score between the initial model and the model plus or minus the effect being tested. I used these results as a Chi-square value with df equal to the number of parameters changed between the two models (Pinheiro and Bates 2000; West et al. 2007). I used both forward and backward model selection procedures to determine the best model fit. In all cases, both procedures resulted in the same model. To determine whether the main effects should be included in the model, I used maximum likelihood rather than restricted maximum likelihood while performing LRT, because the penalty term associated with the restricted maximum likelihood depends on the main effects (Pinheiro and Bates 2000; West et al. 2007). Furthermore, I tested for interactions and none were significant ($p < 0.1$).

Because aggression and boldness are expected to be correlated, I further tested for the presence of behavioral syndromes. Using nestlings that could be assigned personality types (proactive or reactive) I performed a Chi Square test to determine the likelihood of each personality type occurring at high- and low-density breeding sites. Next, I ran correlations between nestling behaviors (aggression and breathing rate) to determine whether aggression and boldness were correlated. Because correlations between behaviors should indicate an adaptation to the environment (Svensson et al. 2001; Dingemanse and Reale 2005; Bell and Sih 2007; Dingemanse et al. 2007), I further analyzed correlations by grouping nestlings based on whether they were hatched and raised in the same environment (not swapped) or if they were raised in an environment other than the one in which they were hatched (swapped). Because nestling behaviors were measured at 14 days, and the environment they experienced is primarily controlled by the parents raising them, I also tested the influence of parental feeding rates (one of the few interactions nestlings have with their parents) on these behaviors. Furthermore, because yolk T influences metabolism (Tobler et al. 2007), females may deposit yolk T to not only prepare offspring for a particular environment but also for a provisioning rate. Therefore, for nestlings that were reared in an environment other than where they were hatched, the difference in feeding rate was calculated as a difference between the rate at which their biological and foster parents fed.

RESULTS

Density Manipulation

Different densities existed within the high- and low-density sites throughout the breeding season. Yolk T concentrations significantly increased at greater densities ($r^2 = 0.22$, $F_{1,28} = 7.47$, $p = 0.01$). The sites designated as low density ultimately had an average density of (mean +/- SE) 0.02 +/- 0.001 pairs/m resulting in an average of 6 pairs and a range of 4 to 8 pairs per 300 m radius during the breeding cycle. The site designated as high density had an average density of 0.09 +/- 0.001 pairs/m resulting in an average of 27 pairs and a range of 7 to 28 pairs per 300 m radius, which was significantly more than the low-density sites (table 1). Site density significantly influenced the number and type of interactions. There were significantly more interactions at the high-density site (4.66 +/- 0.57) than at the low-density sites (2.34 +/- 0.70; $F_{1,15} = 25.92$, $p < 0.001$; table 2). Birds nesting in the high-density site employed significantly more vocal rather than physical interactions (-1.54 +/- 0.34), while birds nesting at low-density sites used more physical than vocal interactions (0.30 +/- 0.42; $F_{1,15} = 7.81$, $p = 0.004$; table 2). Female age was not significantly different between the two sites ($\chi^2 = 2.40$, df = 1, $p = 0.30$). However, boxes settled at the high-density site had a significantly higher habitat suitability score (table 1). Yolk T was significantly higher at the high-density (2.61 +/- 0.28 ng/g) than the low-density sites (1.34 +/- 0.26 ng/g; $F_{2,\,16} = 3.17$, $p = 0.05$; table 3; fig. 4). No significant model predicted variation in yolk corticosterone ($F_{1,18} = 1.89$, $p = 0.17$); in this population the mean yolk corticosterone concentration was 1.38 +/- 0.33 ng/g.

Nestling Physiology

The H/L ratio of siblings raised in the same nest was not significantly different from their fostermates in a paired *t*-test ($t_{2,18}$ = -0.19, p = 0.85), suggesting density did not influence this measure of stress and health. Growth rate of siblings raised in the same nest was significantly different from their fostermates in a paired *t*-test; nestlings hatched at the high-density site (2.17 +/- 0.05 g/d) had significantly higher growth rates than their fostermates hatched at low-density sites (2.07 +/- 0.04 g/d; $t_{2,18}$ = 2.37, p = 0.029; fig. 5a).

Mixed model analysis indicated that the H/L ratio was not influenced by density at hatch site, but rather brood size. H/L ratios were higher at higher brood sizes ($F_{1,59}$ = 9.60, p = 0.003, table 4; fig. 6), suggesting that nestling exhibited better heath in smaller broods. Random factors were removed from the H/L ratio model, because neither nest of origin (Z = 1.16, p = 0.25) nor nest reared (Z = 0.06, p = 0.95) explained a significant proportion of the variance in the model and the model was not significantly improved by including the random effects (χ^2 = -3.095, df = 2, p = 0.21). Repeated measures mixed models showed that yolk corticosterone levels were negatively related to mass at fledging age ($F_{1,19}$ = 5.21, p = 0.03; table 5; fig. 7a). Additionally, nestlings that were hatched at the high-density site exhibited greater mass gain during the nestling stage ($F_{1,35}$ = 6.28, p = 0.017; table 5; fig. 7b). Nestling mass also significantly increased with age ($F_{4,105}$ = 3176.39, p < 0.001); however, that is to be expected. Both nest of origin and nest reared were included as random factors, because nest reared (Z = 1.69, p = 0.09) and nest of origin (Z = 1.80, p = 0.07) were important contributors to the model and significantly improved the model fit (χ^2 = -66.54, df = 2, p < 0.001). Furthermore, nestling aggression significantly influenced the number of feeds they received. Nestlings with high aggression scores received significantly more feeds as they increased their begging rate ($r^2 = 0.16$, $F_{1,35} = 6.55$, $p = 0.02$); whereas, nestlings with low aggression scores did not receive more feeds as they begged more ($r^2 = 0.03$, $F_{1,29} = 0.74$, $p = 0.40$; fig. 8).

Nestling Behavior

Breathing rate during handling stress was highly repeatable with an intraclass correlation coefficient of 0.799, supporting the use of a breathing rate average. Breathing rate score (controlled for by air temperature) of siblings reared in the same nest was not significantly different from their fostermates in a paired *t*-test; nestlings hatched at the high-density site (0.25 +/- 0.23) had a trend to be higher than their fostermates hatched at the low-density sites (-0.31 +/- 0.16; $t_{2,17}=1.91$, $p=0.074$; fig. 5b). Aggression scores of siblings reared in the same nest were significantly different from their fostermates in a paired *t*-test; nestlings hatched at the high-density site (0.32 +/- 0.16) had significantly higher aggression scores than their fostermates hatched at low-density sites (-0.28 +/- 0.21; $t_{2,18} = 4.94$, $p < 0.001$; fig. 5c).

Mixed model analysis indicated that breathing rate during handling stress (controlled for by air temperature) was significantly higher in nestlings hatched in high-density sites (0.43 +/- 0.19) than low-density sites (-0.30 +/- 0.19; $F_{1,51} = 5.93$, $p = 0.018$; table 6; fig. 9a). Random effects were removed because nest of origin explained no variance and nest reared ($Z = 0.29$, $p = 0.77$) was not significant, and they did not significantly improve the model fit ($\chi^2 = -0.10$, df = 2, $p = 0.95$). Mixed model analysis also showed that aggression during feeding was significantly higher in nestlings hatched in the high-density site (0.30 +/- 0.22) compared to low-density sites (-0.25 +/- 0.22; $F_{1,43} = 4.26$, $p = 0.045$; table 7; fig. 9b). Only nest reared ($Z = 1.91$, $p = 0.057$) was included as a random effect, because nest of origin was redundant in that it explained zero variance in the model. Furthermore, including nest reared significantly improved the model fit ($\chi^2 = -8.83$, df = 1, $p = 0.003$).

Of those nestlings that could be confidently categorized as proactive or reactive, personalities did not differ based on site reared ($\chi^2 = 0.01$, df = 1, $p = 0.94$) or swap status ($\chi^2 = 0.01$, df = 1, $p = 0.94$); however, more proactive individuals than were expected hatched at the high-density site and more reactive individuals than expected were hatched at the low-density sites ($\chi^2 = 12.59$, df = 1, $p < 0.001$). Breathing rate score and aggression score were not directly correlated ($r = 0.11$, df = 68, $p = 0.35$). However, the relationship became stronger when the analysis was split by whether or not nestlings were hatched and reared in the same environment. No significant relationship was found between aggression and breathing rate scores for nestlings hatched in one environment and raised in another ($r = 0.02$, df = 33, $p = 0.93$; fig. 10A); however, there was a positive trend among nestlings that were hatched and reared in the same environment ($r = 0.27$, df = 34, $p = 0.118$; fig. 10B). This relationship was clearly driven by the fact that nestlings with high breathing rates never exhibited low aggression scores (fig. 10B). For nestlings that were reared in an environment other than where they were hatched, a correlation of the difference in feeding rate (biological parents feeding rate minus foster parents feeding rate) on breathing rate score ($r = 0.14$, df = 31, $p = 0.47$; fig. 11A) and aggression score ($r = -0.31$, df = 32, $p = 0.08$; fig. 11B) showed that there was a trend for these nestlings to adjust their aggression depending on the feeding rate at the nest they were reared; nestlings were less aggressive if their foster parents fed more often than their biological parents. Independent *t*-tests indicated that parents did not feed nestlings differently based on the site density ($t_{2,30} = -0.27$, $p = 0.79$). Indeed, of the two behaviors I measured, aggression during feeding competition showed more variance than did breathing rate between reactive (N = 15, aggression SE = 0.22, breathing rate SE = 0.12) and proactive (N = 22, aggression SE = 0.09, breathing rate SE = 0.12) individuals.

DISCUSSION

Density Manipulation

The nest box manipulation created breeding sites that significantly differed in levels of density. Female tree swallows nesting in the "high density" site in which nest boxes were only 12 m apart, experienced a significantly higher population density during breeding than females nesting in "low density" sites in which boxes were at least 36 m apart. Contrary to Male et al. (2006), I did not find a significant difference in first egg dates or clutch sizes between the high and low-density sites; in fact, no reproductive parameters differed between the sites. Nest boxes at the high-density site did have a significantly higher habitat suitability score; however, this variable did not contribute significantly to any of the models predicting either reproductive performance, yolk hormones, or nestling phenotype. This could be because the average habitat suitability score was only 3 points different between the high- and low-density sites, which may not be enough to affect reproductive parameters or nestling phenotype.

Furthermore, I found that females that bred at high- and low-density sites experienced different levels and types of aggressive interactions with conspecifics. Overall, I observed a greater number of aggressive interactions at the high- than low-density sites and my data corroborate those of Male et al. (2006). Interestingly, pairs in high- and low-density sites exhibited aggressive behavior differently. Birds at the high-density site generally responded to conspecific intrusions with aggressive vocalizations; whereas, birds in low density responded more often with physical chasing. While physical chasing may appear to be a stronger manifestation of aggression, this is unlikely because low-density pairs were not more aggressive. Indeed, recent literature suggests that vocalizations are more tightly controlled by circulating T levels than are physical interactions (Levin and Wingfield 1992; Brenowitz and Kroodsma 1996; Van Duyse et al. 2002; Soma 2006). Van Duyse et al. (2002) found that vocalizations toward a decoy were significantly increased with T implants, but physical aggression was not. It may not be necessary to have high T levels to also be more physically aggressive and many studies have shown that aggression can occur without high T (Silverin 1993; Wikelski et al. 1999). Van Duyse et al. (2002) suggest that breeding baseline T levels are enough to elicit physical aggression and increases in T have no additional effect. Therefore, when aggressive interactions are increased, like in high-density environments, one may expect to see more aggressive vocalizations, but not necessarily more physical interactions. An increase in vocalizations at the high-density site may better reflect high circulating levels of T in breeding females. However, an alternative explanation is that because birds experience more interactions at high-density sites, they conserve energy by being more selective regarding which intruders to chase.

At the high-density site females laid eggs with significantly higher concentrations of yolk T than low-density sites. Several studies have found that high breeding density increases yolk T (Reed and Vleck 2001; Whittingham and Scwabl 2001; Mazuc et al. 2003; Pilz and Smith 2004; Hargitai et al. 2009). This relationship is commonly attributed to increased aggression in high-density environments and my data corroborate this. Furthermore, the mean yolk T value from my high-density site is similar to those documented in female tree swallows by Whittingham and Schwabl (2001) at a high-aggression breeding site. These data show that my breeding site density manipulation successfully altered levels of social aggression and naturally caused higher yolk T concentrations in high-density clutches.

I failed to find a significant model predicting concentrations of yolk corticosterone. Love et al. (2008) showed that yolk corticosterone concentrations were lower in high-density environments. The authors suggest that semi-colonial birds that nest at higher densities are able to increase predator vigilance and lower stress. However, I found no influence of density on yolk corticosterone. It may be that my sites are not suffering from heavy predation; only 1 nest was destroyed by a predator. Additionally, the yolk corticosterone concentrations in my population were low compared to other studies of wild bird populations (Love et al. 2008; Navara et al. 2006b). These data suggest my population may not have been experiencing particularly stressful conditions.

Nestling Physiology

Nestling H/L ratio was not significantly influenced by breeding site density. The absence of a relationship between H/L ratio and field site density indicates that the sites did not cause different levels of stress. When experiencing stressors, heterophils increase and lymphocytes decrease leading to higher H/L ratios (Brown 1996). The H/L ratio is well-suited to reflect long term stressors, such as poor environment, because leukocytes respond more slowly to stress than transient hormones (Gross and Siegel 1983; Brown 1996; Vleck et al. 2000; Muller et al. 2011). My failure to detect a difference in H/L ratios associated with field site further suggests that the difference in habitat suitability I found between my high- and low-density field sites did not affect nestling physiology.

Alternatively, the absence of a relationship between H/L ratio and field site also indicates that hatch site density did not influence overall health. Other studies show that nestlings exposed to elevated yolk T have a compromised cellular immune response (Navara et al. 2005), superior cellular immune response (Navara et al. 2006a), or no change in immunity (Rubolini et al. 2006; Pitala et al. 2009). Specifically, Navara et al. (2005) and Gil et al. (2006) found no influence of yolk androgens on H/L ratio. One may expect increased T to increase the H/L ratio, because T causes oxidative stress (von Schantz et al. 1999), which damages lymphocytes leading to immunosuppression and a higher H/L ratio (Raberg et al. 1998). However, Braude et al. (1999) proposed that changes in leukocytes arising from increased T result from immunoredistribution rather than immunosuppression. Leukocytes may migrate from circulation to lymph nodes, skin, and other tissues making it difficult to detect changes in immunity using H/L ratios (Braude et al. 1999). I did not asses other measures of cellular or humoral immunity. If I had, I may have found a more direct relationship, because higher concentrations of T have been shown to cause apoptosis in thymic cells (Olsen et al. 1991) and hinder development of the bursa of Fabricus, the site of B cell maturation (Glick 1986).

I did find a positive relationship between H/L ratio and brood size, which was also found by Ilmonen et al. (2003). Larger broods are likely a source of more nutritional and social stress (Kozlowski and Ricklefs 2011); thereby, causing an increase in H/L ratio. Because brood size negatively influenced H/L ratios, it is likely that the detrimental effects of yolk hormones on H/L ratios would have been detected, had they occurred.

Hatch site density did influence nestling growth; nestlings grew significantly faster when they hatched from high-density sites, regardless of rearing environment. Faster growth as a result of increased prenatal T exposure has been found by numerous studies using egg injections (Schwabl 1996b; Pilz et al. 2004; Navara et al. 2005; Navara et al. 2006a; Muller et al. 2007). The positive effect of early T exposure on growth is likely mediated by an increase in food consumption. Higher yolk T levels can increase metabolic rates (Tobler et al. 2007), which could cause nestlings to have a higher food demand, leading to an increase in begging. Accordingly, studies have found that increased exposure to prenatal T causes more begging (Eising and Groothuis 2003; von Engelhardt et al. 2006). I found a positive, non-significant trend between begging rate and nestling growth. Furthermore, nestlings from high-density environments were more aggressive and high-aggression nestlings begged more efficiently, thus, receiving more food as they increased begging rate. These data suggest that competitiveness during begging may be the mechanism responsible for increased growth in nestlings hatched from high T egg yolks.

Yolk corticosterone had a negative effect on nestling growth; nestlings hatched from eggs with high yolk corticosterone fledged with significantly lower body mass. These data are consistent with other studies (Hayward and Wingfield 2004; Saino et al. 2005; Wada and Breuner 2008). Yolk corticosterone influences growth similarly to yolk testosterone, however, elevated yolk corticosterone decreases begging rates (Rubolini et al. 2005; Wada and Breuner 2008). A decrease in begging rate could cause a lower final mass in the same way that an increase in begging rate can cause a higher mass. It is also interesting to note that yolk corticosterone exerted its effect on growth much later in development than yolk T. This could be because many vertebrates undergo a stress hyporesponsive period during early postnatal development, in which they decrease their sensitivity to stress hormones during periods of critical development (Schapiro et al. 1962; Schmidt et al. 2005; Quillfeldt et al. 2009).

Nestling Behavior

Breathing rate during handling stress was significantly higher in nestlings hatched in high- compared to low-density sites. Although breathing rate is a relatively untested measure of boldness and has produced controversial results, there are few methodologies for measuring boldness in altricial nestlings. For example, Carere and van Oers (2004) found that shyer nestlings had higher breathing rates during handling stress. However, more recent studies have found the opposite effect. Fucikova et al. (2009) found that more exploratory nestlings had higher breathing rates during handling stress and Bell et al. (2010) observed higher respiration rates in three-spined sticklebacks (*Gasterosteus aculeatus*) that were more aggressive and bold. Alternatively, in adult female zebra finches, boldness and breathing rate are not correlated (David et al. 2012). Nevertheless, it would stand to reason that breathing rate should be higher in bolder, proactive individuals. Proactive individuals tend to have higher levels of sympathetic activity ("fight or flight"); whereas, reactive individuals have higher parasympathetic activity ("rest and digest"; Siegel 1980; Koolhaas et al. 1999). When under stress, such as manual restraint, proactive individuals react with a stronger sympathetic response by actively trying to escape causing increases in heart rate, oxygen consumption, and breathing rate. Reactive individuals, on the other hand, have hormonal mechanisms allowing them to adjust to stress passively (Koolhaas et al. 1999; Korte et al. 1999; Arens and Cooper 2005; Careau et al. 2008). Indeed, in tonic immobility tests, shy individuals are more likely to be induced to immobility (Daisely et al. 2005). Therefore, it seems reasonable to infer that nestlings in my study that were exposed to higher levels of yolk T are bolder individuals, because they react to stress with a more active sympathetic response (higher breathing rates).

Aggression during feeding was also significantly greater in nestlings hatched from high- compared to low-density sites regardless of rearing environment. Studies that experimentally enhanced yolk T concentrations show that T can cause more aggressive behaviors in nestlings (Eising et al. 2006; Muller et al. 2009). My measures of aggression included begging posture and position in the nest during feeding. Enhanced begging posture could be facilitated by yolk T due to its positive effect on the development of neck muscles (Lipar and Ketterson 2000). However, oral T administration to nestlings also increases height of begging posture (Goodship and Buchanan 2007), suggesting enhanced muscles are not needed. Alternatively, increased yolk T increases metabolic rate (Tobler et al. 2007), which could increase a need for food and lead to increased begging vigor. In terms of nest position, nestlings have been shown to position themselves in the nest where they predict parents will feed (Gottlander 1987; McRae et al. 1993; Kollilker and Richner 2004). Increased yolk T experienced by nestlings at the high-density site likely helped them compete for these positions by increasing their metabolism and activity levels (Eising et al. 2006; Muller et al. 2009). Kolliker and Richner (2004) found that great tit nestlings that position themselves have an 8% competitive advantage over randomly moving siblings (0.4 feeds more per hour). I did see that more aggressive nestlings begged more efficiently than nestlings with low aggression scores, as indicated by the positive significant relationship between begging rate and feeds received in high- but not low-aggression nestlings.

It is intriguing to postulate that increased yolk T concentrations in response to high-density environments are an adaptation to facilitate a competitive advantage (higher aggression and boldness) for fledglings or for nestlings returning to higher density sites as adults. Unfortunately, it is difficult to measure the adaptive value of these behavioral traits, because it is not logically feasible to measure lifetime reproductive success or survivorship for these nestlings. However, recent literature argues that if behaviors are adaptive for particular environments they are expected to be correlated and to represent behavioral syndromes (proactive and reactive). It stands to reason that, for correlational selection to occur between two traits, there must be some selection pressure favoring both traits (Svensson et al. 2001; Dingemanse and Reale 2005). Indeed, boldness and aggression in sticklebacks were only correlated when individuals were under predation pressure (Huntingford 1976; Bell and Sih 2007; Dingemanse et al. 2007). Therefore, I categorized nestlings as either proactive (positive scores for both behaviors) or reactive (negative scores) and found that proactive individuals were significantly more likely to have hatched from the high-density site than expected. However, my measures of boldness and aggression were not directly correlated. Nevertheless, when I separated nestlings into those that were hatched and reared in the same environment from those that were swapped between sites, I found a trend for aggression and boldness to be positively related in nestlings that were hatched and reared in the same site. However, I found none such relationship among nestlings that were hatched and reared in different environments (swapped nestlings). The tendency for nestling behaviors to be more strongly correlated when nestlings are hatched and reared in the same environment is expected if prenatal hormones allow offspring to be better adapted to the environment that the mother experiences during egg yolking. However, when nestlings are moved from a high- to low-competitive environment (or vice versa) their behaviors may be inappropriate for the new environment. My data lend some support to the idea that females can create adaptive behavioral phenotypes for an anticipated environment using hormonal provisioning.

The finding that behaviors are more correlated in nestlings that remained in their natal environment begs the question: why are aggression and boldness not correlated when nestlings are swapped? Of the two behaviors, aggression showed more variation than boldness, a finding shared by Bell and Sih (2007). In particular, reactive nestlings exhibited more variation in aggression. These data are consistent with previous studies showing that reactive individuals have a higher level of behavioral plasticity, because as slower explorers, they pay more attention to their surroundings and can identify changes more readily (Verbeek et al. 1999; Guillette et al. 2011). Therefore, it is more likely that nestlings can alter their aggressiveness during feeding in relation to hunger rather than the complex cascade of events triggered by a sympathetic response to stress (Koolhaas et al. 1999; Korte et al. 2005). More-competitive nestlings should beg less when satiated and beg more vigorously when hungry (Price et al. 2002). Moreover, yolk T influences metabolic rates (Tobler et al. 2007) and females may use hormonal provisioning to metabolically prepare offspring for a level of provisioning they are capable of providing. To test this idea, I examined the response of nestlings that were swapped to the feeding rate they received from foster parents as opposed to the feeding rate they biologically expected. While I did not find a site difference in feeding rates, the difference between what feeding rate nestlings were prepared to receive (biological parents' feeding rate) and what they actually received from foster parents seemed to affect their aggression. Nestlings that were fed more often by foster parents than would be expected by their biological parents exhibited lower aggression scores, while those fed less often had higher aggression scores. This variation in aggression may have caused the decoupling of the behaviors in nestlings that were swapped. Maternal effects may allow offspring to display traits that would allow them to be particularly fit for the competitive environment that the mother experiences and the provisioning rates that the biological parents are likely to provide. However, if nestlings are reared in an environment where foster parents feed at a higher than expected rate, they may reduce unnecessary competitive behaviors.

Behavioral plasticity in aggression may lead to personalities not associated with behavioral syndromes, which generally only encompass correlated behaviors along the linear proactive-reactive axis. For example, it is possible that the relationship between aggression and boldness exhibited in nestlings that were not swapped is not linear. Indeed, the wedge-shaped data indicate that individuals displaying bold (high sympathetic activity) and non-aggressive behaviors were selected against, while individuals that were bold and aggressive, shy and non-aggressive, and shy and aggressive were positively selected for based on their presence in the population. This selection could be caused by increased energy demands in bold nestlings experiencing high sympathetic reactivity. The sympathetic response mobilizes energy stores (Koolhaas et al. 1999); therefore, bold individuals could be utilizing plasticity in aggression to increase the number of feeds they get to meet these energy demands. If this is true, then bold and non-aggressive nestlings would certainly be selected against, because they would have a net energy loss. Nestlings that are bold would therefore increase their aggression to meet their energy demands and bold-non-aggressive individuals would be nonexistent in the population. Alternatively, bold and aggressive, and shy and non-aggressive nestlings would be able to balance energy demands and shy and aggressive nestlings would have a net energy gain. In fact, it would seem that all individuals would want to be shy and aggressive; however, individual constraints in competitive ability may prevent this.

Conclusions

It is generally thought that maternal effects are shaped by natural selection to act as mechanisms for adaptive phenotypic response to environmental heterogeneity. Maternal effects could be an adaptive means by which a female can change the phenotype of her offspring hormonally in preparation for a given environment (Mousseau and Fox 1998; Groothuis et al. 2005; Carere and Balthazart 2007). This study has shown that high-density environments elicit more social aggression and consequently more yolk T, the nestlings from which grow more quickly and display more aggressive and bold behavioral traits. Moreover, that these behavioral traits were more strongly correlated in nestlings that were hatched and reared in the same environment suggests that these maternal effects create adaptive phenotypes for competitive environments. Future studies should investigate the mechanism responsible for the effects prenatal exposure to yolk T exerts on offspring phenotype. Currently, researchers suspect that the organizational effects of early androgen exposure are mediated by epigenetic effects that alter gene expression, thus, affecting hormone regulation and possibly behaviors (Crews 2008). Furthermore, DNA methylation is thought to be heritable; methylation often occurs at CpG sites associated with the 5’ promoter region of genes and can be imprinted in the germline (Crews 2008). Therefore, epigenetic effects instigated by early hormonal provisioning have the potential to generate evolutionary change.

REFERENCES

Alonso-Alvarez, C., S. Bertrand, B. Faivre, O. Chastel, and G. Sorci. 2007. Testosterone and oxidative stress: the oxidative handicap hypothesis. Proceedings of the Royal Society B 274:819-825.

Andersson, S., T. Uller, M. Lohmus, and F. Sundstrom. 2004. Effects of egg yolk testosterone on growth and immunity in a precocial bird. Journal of Evolutionary Biology 17:501-505.

Arens, J.R., and S.J. Cooper. 2005. Metabolic and ventilator acclimatization to cold stress in house sparrows (*Passer domesticus*). Physiological and Biochemical Zoology 78:579-589.

Bell, A.M., and A. Sih. 2007. Exposure to predation generates personality n threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters 10:828-834.

Bell, A.M., L. Henderson, and F.A. Huntingford. 2010. Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. Journal of Comparative Physiology B 180:211-220.

Bernardo, J. 1996. Maternal effects in animal ecology. American Zoology 36:83-105.

Braude, S., Z. Tang-Martinez, and G.T. Taylor. 1999. Stress, testosterone, and the immunoredistribution hypothesis. Behavioral Ecology 10:345-350.

Brenowitz, E.A., and D.E. Kroodsma. 1996. The neuroethology of birdsong. Pages 285-304 *in* D.E. Kroodsma and E.H. Miller, eds. Ecology and evolution of acoustic communication in birds. Cornell University Press.

Brown, M.E. 1996. Assessing body condition in birds. Pages 67-135 *in* V. Nolan Jr. and E.D. Ketterson eds. Current ornithology. Plenum, New York.

Brown, C.R. 1997. Purple martin (*Progne subis*). *In* A. Poole, ed. The birds of North American online. Ithaca: Cornell Lab of Ornithology; Retrieved from the birds of North America online: http://bna.birds.cornell.edu/bna/species/287.

Brown, C.R., and M.B. Brown. 1999. Barn Swallow (*Hirundo rustica*). *In* A. Poole, ed. The Birds of North America Online. Ithaca: Cornell Lab of Ornithology; Retrieved from The Birds of North America Online: http://bna.birds.cornell.edu/bna/species/452

Careau, V., D. Thomas, M.M. Humphries, and D. Reale. 2008. Energy metabolism and animal personality. Oikos 117:641-653.

Carere, C., and L. van Oers. 2004. Shy and bold great tits (*Parus major*): body temperature and breath rate in response to handling stress. Physiology and Behavior 82:905-912.

Carere, C., and J. Balthazart. 2007. Sexual versus individual differentiation: the controversial role of avian maternal hormones. Trends in Ecology and Evolution 18:73-80.

Crews, D. 2008. Epigenetics and its implications for behavioral neuroendocrinology. Frontiers in Neuroendocrinology 29:344-357.

Daisley, J.N., V. Bromundt, E. Mostl, and K. Kotrschal. 2005. Enhanced yolk testosterone influences behavioral phenotype independent of sex in Japanese quail chicks *Coturnix japonica*. Hormones and Behavior 47:185-194.

David, M., Y. Auclair, F. Dechaume-Moncharmont, and F. Cezilly. 2012. Handling stress does not reflect personality in female zebra finches (*Taeniopygia guttata*). Journal of Comparative Psychology 126:10-14.

Davis, A.K., K.C. Cook, and S. Altizer. 2004. Leukocyte profiles in wild house finches with and without Mycoplasmal conjunctivitis, a recently emerged bacterial disease. Ecohealth 1:362-373.

Dein, F.J. 1986. Chemical avian medicine and surgery. Pages 174-191 *in* L.A. Harrison, ed. Hematology. Saunders, Philadelphia.

Dingemanse, N.J., and D. Reale. 2005. Natural selection and animal personality. Behaviour 142:1165-1190.

Dingemanse, N.J., J. Wright, D.K. Thomas, R. Hickling, and N. Dawnay. 2007. Behavioural syndromes differ predictably between twelve populations of stickleback. Journal of Animal Ecology 76:1128-1138.

Drent, P.J., K. van Oers, and A.J. van Noordwijk. 2003. Realized heritability of personalities in the great tit (*Parus major*). Proceedings of the Royal Society B 270:45-51.

Duckworth, R.A. 2010. Evolution of personality: developmental constraints on behavioral plasticity. The Auk 127:752-758.

Dufty, A.M., J. Clobert, and A.P. Moller. 2002. Hormones, developmental plasticity and adaptation. Trends in Ecology and Evolution 17:190-196.

Dunn, P.O., and S.J. Hannon. 1991. Intraspecific competition and the maintenance of monogamy in tree swallows. Behavioral Ecology 2:258-266.

Eising, C.M., and T.G.G. Groothuis. 2003. Yolk androgens and begging behavior in black-headed gull chicks: an experimental field study. Animal Behavior 66:1027-1034.

Eising, C.M., W. Muller, and T.G.G. Groothuis. 2006. Avian mothers create different phenotypes by hormone deposition in their eggs. Biology Letters 2:20-22.

Elf, P.K., and A.J. Fivizzani. 2002. Changes in sex steroid levels in yolks of the leghorn chicken, *Gallus domesticus*, during embryonic development. Journal of Experimental Zoology 293:594-600.

Fidler, A.E., K. van Oers, P.J. Drent, S. Kuhn, J.C. Mueller, and B. Kempenaers. 2007. Drd4 gene polymorphisms are associated with personality variation in a passerine bird. Proceedings of the Royal Society 274:1685-1691.

Finch, D.M. 1990. Effects of predation and competitor interference on nesting success of house wrens and tree swallows. The Condor 92:674-687.

Folstad, I., and A.J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. American Naturalist 139:603-622.

Fucikova, E., P.J. Drent, N. Smite, and K. van Oers. 2009. Handling stress as a measurement of personality in great tit nestlings (*Parus major*). Ethology 115:366-374.

Gil, D., P. Ninni, A. Lacroix, F. de Lope, C. Tirard, A. Marzal, and A.P. Moller. 2006. Yolk androgens in the barn swallow (*Hirundo rustica*): a test of some adaptive hypotheses. Journal of Evolutionary Biology 19:123-131.

Gil, D. 2008. Hormones in avian eggs: physiology, ecology and behavior. Pages 337-398 *in* H.J. Brockmann, T.J. Roper, M. Naguib, K.E. Wynne-Edwards, C. Barnard, and J.C. Mitani, eds. Advances in the study of behavior. Elsevier, Amsterdam.

Glick, B. 1986. The bursa of Fabricius. Pages 87-101 *in* P.D. Sturke, ed. Avian Physiology. Springer-Verlag, New York.

Goodship, N.M., and K.L. Buchanan. 2007. Nestling testosterone controls begging behavior in the pied flycatcher, *Ficedula hypoleuca*. Hormones and Behavior 52:454-460.

Gosling, S.D., and O.P. John. 1999. Peronality dimensions in nonhuman animals: a cross-species review. Current Directions in Psychological Science 8:69-75.

Gottlander, K. 1987. Parental feeding behaviour and sibling competition in the Pied Flycatcher *Ficedula hypoleuca*. Ornis Scandinavica 18:269-276.

Gowaty, P.A., and S.J. Wagner. 1988. Breeding-season aggression of female and male eastern bluebirds (*Sialis sialis*) to models of potential conspecific and interspecific egg dumpers. Ethology 78:238-250.

Groothuis, T.G.G. 1992. The influence of social experience on the development and fixation of the form of displays in the black-headed gull. Animal Behavior 43:1-14.

Groothuis, T.G.G., and H. Schwabl. 2008. Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? Philosophical Transactions of the Royal Society B 363:1647-1661.

Groothuis, T.G.G., and N. von Engelhardt. 2005. Investigating maternal hormones in avian eggs: measurement, manipulation, and interpretation. Annals of the New York Academy of Sciences 1046:168-180.

Groothuis, T.G., W. Muller, N. von Engelhardt, C. Carere, and C. Eising. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neuroscience and Biobehavioral Reviews 29:329-352.

Gross, W.B., and H.S. Siegel. 1983. Evaluation of heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Guillette, L.M., A.R. Reddon, M. Hoeschele, and C.B. Sturdy. 2011. Sometimes slower is better: slow-exploring birds are more sensitive to changes in a vocal discrimination task. Proceedings of the Royal Society B 278:767-773.

Hargitai, R., K.E. Arnold, M. Herenyi, J. Prechl, and J. Torok. 2009. Egg composition in relation to social environment and maternal physiological condition in the collared flycatcher. Behavioral and Ecological Sociobiology 63:869-882.

Hartmann, C., and M. Wilhelmson. 2003. The hen’s egg yolk: a source of biologically active substances. Animal Husbandry 57:477-482.

Hayward, L.S., and J.C. Wingfield. 2004. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. General and Comparative Endocrinology 135:365-371.

Henriksen, R., T.G. Groothuis, and S. Rettenbacker. 2011. Elevated plasma corticosterone decreases yolk testosterone and progesterone in chickens: linking maternal stress and hormone-mediated maternal effects. PLoS ONE 6:e23824.

Hessing, M.J.C., A.M. Hagelso, W.G.P. Schouten, P.R. Wiepkema, and J.A.M. van Beek. 1994. Individual behavioral and physiological strategies in pigs. Physiology and Behavior 55:39-46.

Hinde, C.A., K.L. Buchanan, and R.M. Kilner. 2009. Prenatal environmental effects match offspring begging to parental provisioning. Proceedings of the Royal Society B 276:2787-2794.

Huntingford, F.A. 1976. The relationship between anti-predator behavior and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Animal Behavior 24:245-260.

Hussell, D.J.T. 1983. Age and plumage color in female tree swallows. Journal of Field Ornithology 54:312-318.

Ilmonen, P., D. Hasselquist, A. Langefors, and J. Wiehn. 2003. Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. Oecologia 136:148-154.

Jawor, J.M., J.W. McGlothlin, J.M. Casto, T.J. Greives, E.A. Snajdr, G.E. Bentley, and E.D. Ketterson. 2007. Testosterone response to GnRH in a female songbird varies with stage of reproduction: implication for adult behavior and maternal effects. Functional Ecology 21:761-775.

Kassel, J., and R.E. Davis. 1975. Early behavioral experience and adult social behavior in the paradise fish, *Macropodus opercularis* L. Behavioral Biology 15:343-351.

Kolliker, M., and H. Richner. 2004. Navigation in a cup: chick positioning in great tit, *Parus major*, nests. Animal Behavior 68:941-948.

Koolhaas, J.M., S.M. Korte, S.F. de Boer, B.J. van Der Vegt, C.G. van Reenen, H. Hopster, I.C. de Jong, M.A.W. Ruis, and H.J. Blokhuis. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23:925-935.

Korte, S.M., W. Ruesink, and H.J. Blokuis. 1999. Heart rate variability during manual restrain in chicks from high- and low-feather pecking lines of laying hens. Physiology and Behavior 65:649-652.

Korte, S.M., J.M. Koolhaas, J.C. Wingfield, and B.S. McEwen. 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neuroscience and Biobehavioral Reviews 29:3-38.

Kozlowski, C.P., and R.E. Ricklefs. 2011. The effects of brood size on growth and steroid hormone concentrations in nestling eastern bluebirds (*Sialis sialis*). General and Comparative Endocrinology 173:447-453.

Kurtz, J., K. Reinhold, and L. Engqvist. 2000. Immunosuppression under stress: necessary for condition-dependent signaling? Trends in Ecology and Evolution 15:418-419.

Lessells, C.M., and P.T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. The Auk 104:116-121.

Levin, R.N., and J. Wingfield. 1992. The hormonal control of territorial aggression in tropical birds. Ornis Scandinavica 23:284-291.

Lipar, J.L., and E.D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelaius phoeniceus*. Proceedings of the Royal Society B 267:2005-2010.

Love, O.P., K.E. Wynne-Edwards, L. Bond, and T.D. Williams. 2008. Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. Hormones and Behavior 53:104-111.

Lovern, M.B., and J. Wade. 2003. Sex steroids in green anoles (*Anolis carolinensis*): uncoupled maternal plasma and yolking follicle concentrations, potential embryonic steroidogenesis, and evolutionary implications. General Comparative Endocrinology 134:109–15.

Male, S.K., J. Jones, and R.J. Robertson. 2006. Effects of nest-box density on the behavior of tree swallows during nest building. Journal of Field Ornithology 77:61-66.

Marrone, B.L., and F. Hertelendy. 1983. Steroid metabolism by avian ovarian cells during follicular maturation. Biology of Reproduction 29:953-962.

Marshall, D.J., and T. Uller. 2007. When is a maternal effect adaptive? Oikos 116:1957-1963.

Mazuc, J., C. Bonneaud, O. Chastel, and G. Sorci. 2003. Social environment affects female and egg testosterone levels in the house sparrow (*Passer domesticus*). Ecology Letters 6:1084-1090.

McCarty, J.P., and D.W. Winkler. 1999. Foraging ecology and diet selectivity of tree swallows feeding nestlings. The Condor 101:246-254.

McRae, S.B., P.J. Weatherhead, and R. Montgomerie. 1993. American robin nestlings compete by jockeying for position. Behavioral and Ecological Sociobiology 33:101-106.

Moller, A.P. 1987. Intraspecific nest parasitism and antiparasite behavior in swallows, *Hirundo rustica*. Animal Behavior 35:247-254.

Moller, A.P., and M. Petrie. 2002. Condition dependence, multiple sexual signals, and immunocompetence in peacocks. Behavioral Ecology 13:248-253.

Moretz, J.A., E.P. Martins, and B.D. Robison. 2007. Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behavioral Ecology 18:556-562.

Mousseau, T.A., and C.W. Fox. 1998. The adaptive significance of maternal effects. Trends in Ecology and Evolution 13:403-407.

Muldal, A., H.L. Gibbs, and R.J. Robertson. 1985. Preferred nest spacing of an obligate cavity-nesting bird, the tree swallow. The Condor 87:356-363.

Muller, W., K. Deptuch, I. Lopez-Rull, and D. Gil. 2007. Elevated yolk androgen levels benefit offspring development in a between-clutch context. Behavioral Ecology 18:929-936.

Muller, W., C. Dijkstra, and T.G.G. Groothuis. 2009. Maternal yolk androgens stimulate territorial behavior in black-headed gull chicks. Biology Letters 5:586-588.

Muller, C., S. Jenni-Eiermann, and L. Jenni. 2011. Heterophils/lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings. Functional Ecology 25:566-576.

Munro, H.L., and R.C. Rounds. 1985. Selection of artificial nest sites by five sympatric passerines. Journal of Wildlife Management 49:264-276.

Navara, K.J., G.E. Hill, and M.T. Mendonca. 2005. Variable effects of yolk androgens on growth, survival, and immunity in eastern bluebird nestlings. Physiological and Biochemical Zoology 78:570-578.

Navara, K.J., G.E. Hill, and M.T. Mendonca. 2006a. Yolk testosterone stimulates growth and immunity in house finch chicks. Physiological and Biochemical Zoology 79:550-555.

Navara, K.J., L.M. Siefferman, G.E. Hill, and M.T. Mendonca. 2006b. Yolk androgens vary inversely to maternal androgens in eastern bluebirds: an experimental study. Functional Ecology 20:449-456.

Okuliarova, M., B. Sarnikova, S. Rettenbacher, P. Skrobanek, and M. Zeman. 2010. Yolk testosterone and corticosterone in hierarchical follicles and laid eggs of Japanese quail exposed to long-term restraint stress. General Comparative Endocrinology 165:91-96.

Oliveira, R.F. 2004. Social modulation of androgens in vertebrates: mechanisms and function. Advances in the Study of Behavior 34:165-238.

Olsen, N.J., M.B. Watson, G.S. Henderson, and W.J. Kovacs. 1991. Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. Endocrinology 129:2471-2476.

Owen, J.C., and F.R. Moore. 2008. Relationship between energetic condition and indicators of immune function in thrushes during spring migration. Canadian Journal of Zoology 86:638-647.

Peters, A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. Proceedings of the Royal Society B 267:883-889.

Pilz, K.M., H.G. Smith, M.I. Sandell, and H. Schwabl. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? Animal Behavior 65:841-850.

Pilz, K.M., and H.G. Smith. 2004. Egg yolk androgen levels increase with breeding density in the European starling, *Sturnus vulgaris*. Functional Ecology 18:58-66.

Pilz, K.M., M. Quiroga, H. Schwabl, and E. Adkins-Regan. 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. Hormones and Behavior 46:179-192.

Pinheiro, J.C., and D.M. Bates. 2000. Mixed-effects models in S and S-Plus. Springer, New York.

Pitala, N., S. Ruuskanen, T. Laaksonen, B. Doligez, B. Tschirren, and L. Gustafsson. 2009. The effects of experimentally manipulated yolk androgens on growth and immune function of male and female nestling collared flycatchers *Ficedula albicollis*. Journal of Avian Biology 40:225-230.

Price, K., R. Ydenberg, and D. Daust. 2002. State-dependent begging with asymmetries and costs: a genetic algorithm approach. Pages 21-42 *in* J. Wright and M.L. Leonard, eds. The Evolution of Begging. Springer, Netherlands.

Quillfeldt, P., M. Poisbleau, O. Chastel, and J.F. Masello. 2009. Acute stress hyporesponsive period in nestlings Thin-billed prions *Pachyptila belcheri*. Journal of Comparative Phyiology A 195:91-98.

Raberg, L., M. Grahn, D. Hasselquist, and E. Svensson. 1998. On the adaptive significance of stress-induced immunosuppression. Proceedings of the Royal Society B 265:1637-1641.

Reed, W.L., and C.M. Vleck. 2001. Functional significance of variation in egg-yolk andorgenes in the American coot. Oecologia 128:164-171.

Rendell, W.B., and R.J. Robertson. 1989. Nest-site characteristics, reproductive success and cavity availability for tree swallows breeding in natural cavities. The Condor 91:875-885.

Ricklefs, R. 1984. Variation in the size and composition of eggs of the European starling. The Condor 86:1-6.

Robertson, R.J., and H.L. Gibbs. 1982. Superterritoriality in tree swallows: a reexamination. The Condor 84:313-316.

Robertson, R.J., B.J. Stutchbury, and R.R. Cohen. 1992. Tree swallow. Pages 1-26 *in* A. Poole, P. Stettenheim, and F. Gill, eds. The birds of North America. The Academy of Natural Sciences, Philadelphia.

Rosvall, K.A. 2008. Sexual selection on aggressiveness in females: evidence from an experimental test with tree swallows. Animal Behavior 75:1603-1610.

Rosvall, K.A. 2011. Maintenance of variation in sexually selected traits in females: a case study using intrasexual aggression in tree swallows *Tachycineta bicolor*. Journal of Avian Biology 42:454-462.

Rubolini, D., M. Romano, G. Boncoraglio, R.P. Ferrari, R. Martinelli, P. Galeotti, M. Fasola, and N. Saino. 2005. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. Hormones and Behavior 47:592-605.

Rubolini, D., M. Romano, R. Martinelli, and N. Saino. 2006. Effects of elevated yolk testosterone levels on survival, growth and immunity of male and female yellow-legged gull chicks. Behavioral and Ecological Sociobiology 59:344-352.

Rutkowska, J., T. Wilk, and M. Cichon. 2007. Androgen-dependent maternal effects on offspring fitness in zebra finches. Behavioral and Ecological Sociobiology 61:1221-1217.

Ruuskanen, S., and T. Laaksonen. 2010. Yolk hormones have sex-specific long-term effects on behavior in the pied flycatcher (*Ficedula hypoleuca*). Hormones and Behavior 57:119-127.

Saino, N., M. Romano, R.P. Ferrari, R. Martinelli, and A.P. Moller. 2005. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. Journal of Experimental Zoology 303A:998-1006.

Salt, G.W. 1964. Respiratory evaporation in birds. Biological Reviews 39:113-136.

Schapiro, S., E. Geller, and S. Eiduson. 1962. Neonatal adrenal cortical response to stress and vasopressin. Experimental Biology and Medicine 109:937-941.

Schmidt, M., S. Levine, M.S. Oitzl, M. van der Mark, M.B. Muller, F. Holsboer, and E.R. de Kloet. 2005. Glucocorticoid receptor blockade disinhibits pituitary-adrenal activity during the stress hyporesponsive period of the mouse. Endocrinology 146:1458-1464.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science 90:11446-11450.

Schwabl, H. 1996a. Environment modifies the testosterone levels of a female bird and its egg. Journal of Experimental Zoology 276:157-163.

Schwabl, H. 1996b. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology 114A:271-276.

Schwabl, H., D.W. Mock, and J.A. Gieg. 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Searcy, W.A. 1988. Do female red-winged blackbirds limit their own breeding densities. Ecology 69:85-95.

Siegel, H.S. 1980. Physiological stress in birds. BioScience 30:529-534.

Sih, A., A.M. Bell, and C. Johnson. 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution 19:372-378.

Sih, A., A.M. Bell, J.C. Johnson, and R.E. Ziemba. 2004b. Behavioral syndromes: an integrative overview. The Quarterly Review of Biology 79:241-277.

Sih, A. 2011. Behavioral syndromes: a behavioral ecologist's view on the evolutionary and ecological implications of animal personalities. Pages 313-336 *in* A. Weiss, J.E. King, and L. Murray, eds. Personality and temperament in nonhuman primates. Springer, New York.

Silverin, B. 1993. Territorial aggressiveness and its relation to the endocrine system in the pied flycatcher. General Comparative Endocrinology 89:206-213.

Soma, K.K. 2006. Testosterone and aggression: berthold, birds and beyond. Journal of Neuroendocrinology 18:543-551.

Strasser, R., and H. Schwabl. 2004. Yolk testosterone organizes behavior and male plumage coloration in house sparrows. Behavioral and Ecological Sociobiology 56:491-497.

Svensson, E., B. Sinervo, and T. Comendant. 2001. Condition genotype-by-environment interaction, and correlational selection in lizard life-history morphs. Evolution 55:2053-2069.

Teather, K.L. 1992. An experimental study of competition for food between male and female nestlings of the red-winged blackbird. Behavioral and Ecological Sociobiology 31:81-87.

Tobler, M., and M. Sandell. 2007. Yolk testosterone modulates persistence of neophobic responses in adult zebra finches, *Taeniopygia guttata*. Hormones and Behavior 52:640-645.

Tobler, M., J. Nilsson, and J.F. Nilsson. 2007. Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. Biology Letters 3:408-410.

van Duyse, E., R. Pinxten, and M. Eens. 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, *Parus major*. Hormones and Behavior 41:178-186.

Verbeek, M.E.M., P. de Goede, P.J. Drent, and P.R. Wiepkema. 1999. Individual behavioural characteristics and dominance in aviary groups of great tits. Behaviour 136:23-48.

Verboven, N., P. Monaghan, D.E. Evans, H. Schwabl, N. Evans, C. Whitelaw, and R.G. Nager. 2003. Maternal condition, yolk androgens and offspring performance: a supplementary feeding experiment in the lesser black-backed gull (*Larus fuscus*). Proceedings of the Royal Society B 270:2223-2232.

Vleck, C.M., N. Vertalino, D. Vleck, and T.L. Butcher. 2000. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living adelie penguins. The Condor 102:392-400.

von Engelhardt, N., C. Carere, C. Dijkstra, and T.G.G. Groothuis. 2006. Sex-specific effects of yolk testosterone on survival, begging and growth of zebra finches. Proceedings of the Royal Society B 273:65-70.

von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist, and H. Wittzell. 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proceedings of the Royal Society B 266:1-12.

Wada, H., and C.W. Breuner. 2008. Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. Journal of Experimental Biology 211:1696-1703.

West, B.T., K.B. Welch, and A.T. Galecki. 2007. Linear mixed models: a practical guide using statistical software. Chapman and Hall, Florida.

Whittingham, L.A., and H. Schwabl. 2001. Maternal testosterone in tree swallow eggs varies with female aggression. Animal Behavior 62:1-5.

Wikelski, M., M. Hau, W.D. Robinson, and J.C. Wingfield. 1999. Seasonal endocrinology of tropical passerines: a comparative approach. Pages 1224-1241 *in* N.J. Adams and R.H. Slotow, eds. Proceedings of the 22nd International Ornithological Congress, Durban. BirdLife South Africa, Johannesburg.

Wingfield, J.C., and D.S. Farner. 1975. The determination of 5 steroids in avian plasma by radioimmunoassay and competitive-protein-binding. Steroids 3:311-327.

Wolf, J.B., and M.J. Wade. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B 364:1107-1115.

TABLES AND FIGURES

TABLES

Table 1. Independent t-test for differences in site parameters.

Factor	**Site**	**Mean (+/-SE)**	**df**	***t***	***p***
Habitat Suitability	High	16.6 (0.60)	-3.71	18	**0.002**
	Low	13.6 (0.54)			
# Tree Swallows	High	27.2 (0.42)	-35.78	18	**<0.001**
	Low	6.0 (0.42)			
Density (pairs/m)	High	0.09 (0.001)	-31.76	18	**<0.001**
	Low	0.02 (0.001)			
First Egg Date (Julian Days)	High	131.40 (2.57)	18	0.31	0.76
	Low	132.60 (2.95)			
Clutch Size	High	5.10 (0.03)	18	0.62	0.55
	Low	5.40 (0.37)			
Female Body Condition	High	0.08 (0.49)	18	-0.08	0.94
	Low	0.03 (0.27)			
Pair Aggression	High	1.60 (0.52)	18	0.74	0.47
	Low	2.10 (0.43)			
Pair Boldness (# Feeds Missed)	High	3.56 (0.84)	17	-0.21	0.83
	Low	3.32 (0.79)			
Feeding Rate (Feeds/min)	High	0.38 (0.05)	17	-0.37	0.72
	Low	0.34 (0.03)			
% Yolk	High	24.39 (0.86)	17	-1.57	0.14
	Low	22.76 (1.94)			

Table 2. ANCOVA analyses of the effect of hatching site density and pair aggression on total number of interactions (vocal plus physical interactions; $F_{1,15} = 25.92$, $p < 0.001$) and type of interactions (vocal minus physical interactions; $F_{1,15} = 7.81$, $p = 0.004$). Interactions were observed during a 20 min period at each site for nests that were 100% complete on 5 different days over a span of 2 weeks. The interaction between site and aggression was removed from both models because $p > 0.4$.The mean (SE) number of interactions in low density (n=6) was 2.34 (0.70) and in high density (n=9) it was 4.66 (0.57). The mean (SE) interaction score (positive means more physical and negative means more vocal interactions) in low density (n=6) was 0.30 (0.42) and in high density (n=9) was -1.54 (0.34).

Factors	**B (SE)**	***F***	***p*-value**
Number of Interactions			
Site Density[1]	4.59 (0.89)	13.32	**0.001**
Aggression	0.039 (0.34)	0.01	0.910
Type of Interactions			
Site Density[1]	-1.16 (0.53)	5.98	**0.016**
Aggression	-0.20 (0.20)	0.94	0.35

[1] Density was coded as 0 for low and 1 for high; 1 is the reference.

Table 3. ANCOVA to test predictors of yolk testosterone (ng/g). No interactions had $p > 0.1$ and were removed. The variables female age, clutch size, and first egg date were also removed (all $p > 0.6$). Overall model: $F_{2,16} = 3.17$, $p = 0.05$. The average yolk testosterone at low density sites was 1.34 +/- 0.26 ng/g and at high density it was 2.61 +/- 0.28 ng/g.

Factors	**B (SE)**	***F***	***p*-value**
Site Hatched[1]	-1.27 (0.46)	7.49	**0.02**
Female Body Condition	0.19 (0.14)	1.94	0.19
Female Aggression	0.19 (0.11)	3.19	0.10
Habitat Suitability	-0.11 (0.10)	1.33	0.27

[1] Density was coded as 0 for low and 1 for high; 1 is the reference.

Table 4. Mixed model analysis of effect of density on nestling H/L ratio. Variance (+/- SE) due to residuals is 0.01 +/- 0.002. The test statistic for factors in the model is F and it is χ^2 for factors excluded from the model. Random effects were removed because they did not explain a significant proportion of the variance in the model; nest of origin (Z = 1.16, p = 0.25) or nest reared is (Z = 0.06, p = 0.95).

Factors	**Denominator df**	**Estimate (SE)**	F/χ^2	***p*-value**
Site Raised[1]	59	-0.003 (0.03)	0.02	0.89
Site Hatched[1]	59	-0.004 (0.02)	0.03	0.87
Brood Size	59	0.07 (0.02)	9.60	**0.003**
*Variables Excluded (Backward)**				
% Yolk			0.01	0.92
Hatch Date			0.10	0.75
Fledging Body Condition			0.12	0.73
Yolk Corticosterone (ng/g)			-0.22	0.64
Habitat Suitability			0.54	0.46
Yolk Testosterone (ng/g)			-0.72	0.40
*Variable Excluded (Forward)***				
Yolk Testosterone (ng/g)			11.38	<0.001
% Yolk			2.64	0.10
Yolk Corticosterone (ng/g)			0.22	0.64
Fledging Body Condition			-0.04	0.84
Hatch Date			-0.16	0.69
Habitat Suitability			-0.28	0.60

[1] Density was coded as 0 for low and 1 for high; 1 is the reference.

* None make model significantly worse (more positive) when removed.

****** None make model significantly better (more negative) when added.

Table 5. Mixed models analysis for repeated measures testing effect of density on nestling growth. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to nest reared is 0.08 +/- 0.05 ($Z = 1.69$, $p = 0.09$), nest of origin is 0.17 +/- 0.09 ($Z = 1.80$, $p = 0.07$), and repeated measure is: age 2 = 0.04 +/-0.01, age 5 = 1.25 +/- 0.24, age 8 = 3.47 +/- 0.65, age 11 = 5.53 +/- 1.04, age 14 = 3.70 +/-0.72 (all $Z>3.58$, $p<0.001$). The mean growth (+/-SE) for nestlings hatched in low density is 13.06 +/- 0.17 g/d and hatched in high density is 13.43 +/- 0.18 g/d.

Factors	**Denominator df**	**Estimate (SE)**	***F*/χ^2**	***p*-value**
Site Hatched[1]	185.7	-0.36 (0.16)	5.28	**0.02**
Site Reared[1]	170.0	0.18 (0.15)	1.31	0.25
Begging Rate	36.6	0.03 (0.02)	3.74	0.06
Yolk Testosterone (ng/g)	9.3	0.11 (0.15)	0.50	0.50
Yolk Corticosterone (ng/g)	18.1	-0.57 (0.23)	5.85	**0.03**
% Yolk	33.9	-0.01 (0.02)	0.28	0.60
Age	100.8	d2 -18.79 (0.26) d5 -13.72 (0.30) d8 -5.62 (0.35) d11 -0.37 (0.40) d14 – [2]	3128.79	**<0.001**
*Variables Excluded (Backward)**				
Begging Success			0.001	0.97
Hatch Date			0.13	0.72
Brood Size			0.30	0.58
Habitat Suitability			0.36	0.55
Feeding Rate			0.79	0.37
*Variables Excluded (Forward)***				
Begging Success			0.00	1.00
Brood Size			-0.17	0.68
Hatch Date			-0.24	0.62
Habitat Suitability			-0.38	0.54
Feeding Rate			-0.79	0.37

Nest of origin and nest reared as random effects.

[1] Density was coded as 0 for low and 1 for high; 1 is the reference.

[2] Measure is redundant.

* None make model significantly worse (more positive) when removed.

****** None make model significantly better (more negative) when added.

Table 6. Mixed model analysis of effect of density on nestling breathing rate (controlled for by air temperature) during handling stress. The test statistic for factors in the model is F and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to residuals is 0.78 +/- 0.16. Random effects were removed because nest of origin explained no variance and nest reared ($Z = 0.29$, $p = 0.77$) was not significant. The mean aggression score (+/-SE) for nestlings hatched in low density is -0.30 +/- 0.19 and hatched in high density is 0.43 +/- 0.19.

Factors	**Denominator df**	**Estimate (SE)**	**F/χ^2**	***p*-value**
Site Hatched[1]	51	-0.73 (0.30)	5.93	**0.018**
Site Reared[1]	51	0.06 (0.24)	0.07	0.79
Yolk Testosterone (ng/g)	51	-0.29 (0.20)	2.14	0.15
Fledging Body Condition	51	0.11 (0.12)	0.85	0.36
*Variables Excluded (Backward)**				
Yolk Corticosterone (ng/g)			0.20	0.65
Brood Size			0.26	0.61
% Yolk			0.29	0.59
Hatch Date			0.33	0.57
Habitat Suitability			0.66	0.42
Parent Boldness			1.74	0.19
*Variables Excluded (Forward)***				
% Yolk			0.00	1.00
Yolk Corticosterone (ng/g)			-0.003	0.96
Brood Size			-0.28	0.60
Hatch Date			-0.39	0.53
Habitat Suitability			-0.53	0.47
Parent Boldness			-1.74	0.19

[1] Density was coded as 0 for low and 1 for high; 1 is the reference.

* None make model significantly worse (more positive) when removed.

****** None make model significantly better (more negative) when added.

Table 7. Mixed model analysis of effect of density on nestling aggression score. The test statistic for factors in the model is *F* and it is χ^2 for factors excluded from the model. The variance (+/- SE) due to nest reared is 0.40 +/- 0.21 (*Z* = 1.91, *p* = 0.057) and residuals is 0.53 +/- 0.12. Nest of origin was excluded because it was redundant and explained zero variance. The mean aggression score (+/-SE) for nestlings hatched in low density is -0.25 +/- 0.22 and hatched in high density is 0.30 +/-0.22.

Factors	**Denominator df**	**Estimate (SE)**	***F*/χ^2**	***p*-value**
Site Hatched[1]	43.0	-0.54 (0.26)	4.26	**0.045**
Site Reared[1]	16.1	0.10 (0.35)	0.08	0.78
Yolk Testosterone (ng/g)	44.9	0.07 (0.18)	0.16	0.69
Fledging Body Condition	49.1	-0.04 (0.13)	0.10	0.75
*Variables Excluded (Backward)**				
Parent Aggression			0.12	0.73
Habitat Suitability			0.28	0.60
% Yolk			0.28	0.60
Hatch Date			0.30	0.58
Brood Size			0.64	0.42
Yolk Corticosterone (ng/g)			0.78	0.38
Feeding Rate			2.65	0.10
*Variables Excluded (Forward)***				
Parent Aggression			-0.001	0.97
Habitat Suitability			-0.05	0.82
Hatch Date			-0.11	0.74
% Yolk			-0.32	0.57
Yolk Corticosterone (ng/g)			-0.40	0.53
Feeding Rate			- 0.47	0.49
Brood Size			-0.64	0.42

Nest reared as random effect.

[1] Density was coded as 0 for low and 1 for high.

* None make model significantly worse (more positive) when removed.

****** None make model significantly better (more negative) when added.

FIGURES

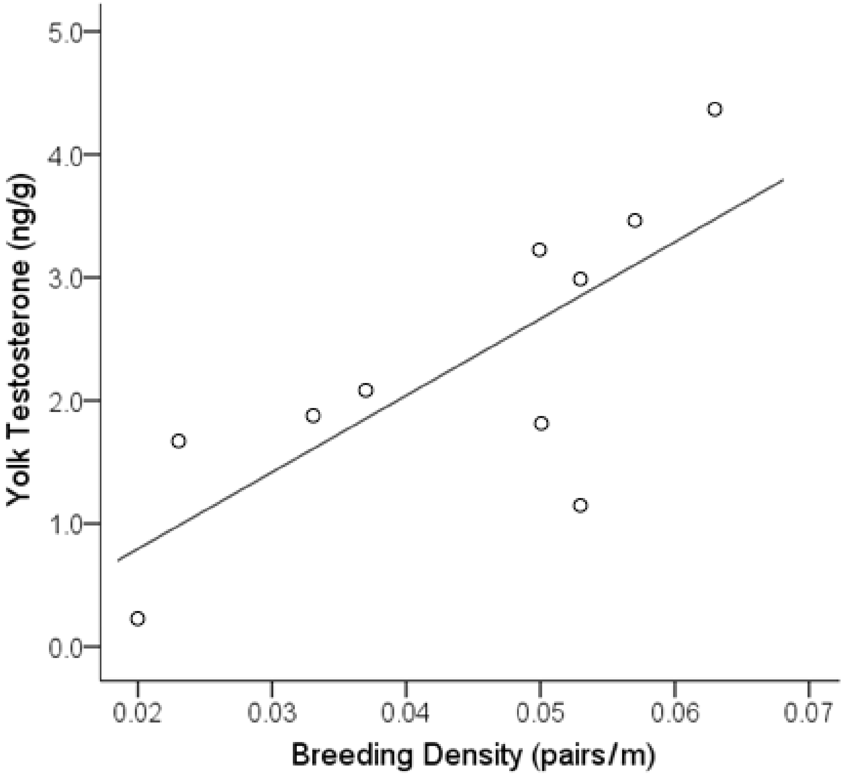

Figure 1. Preliminary data from 2009; regression of average tree swallow breeding density and concentration of yolk testosterone ($r^2 = 0.56$, $F_{1,9} = 10.37$, $p = 0.012$).

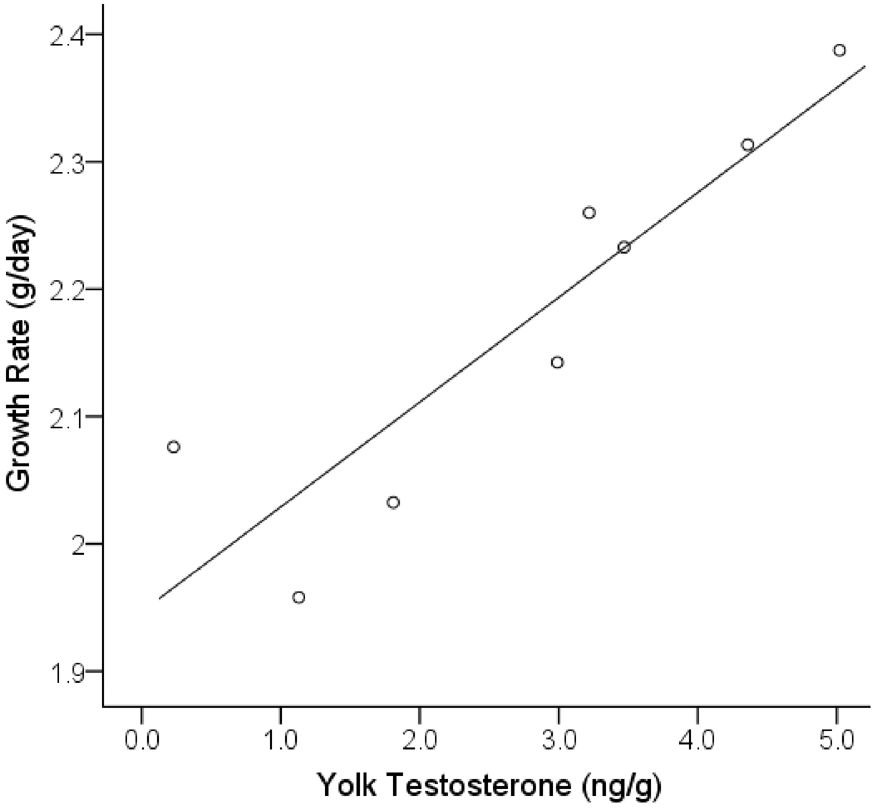

Figure 2. Preliminary data from 2009; regression of yolk testosterone concentrations and average brood growth rate ($r^2 = 0.81$, $F_{1,7} = 26.20$, $p = 0.002$).

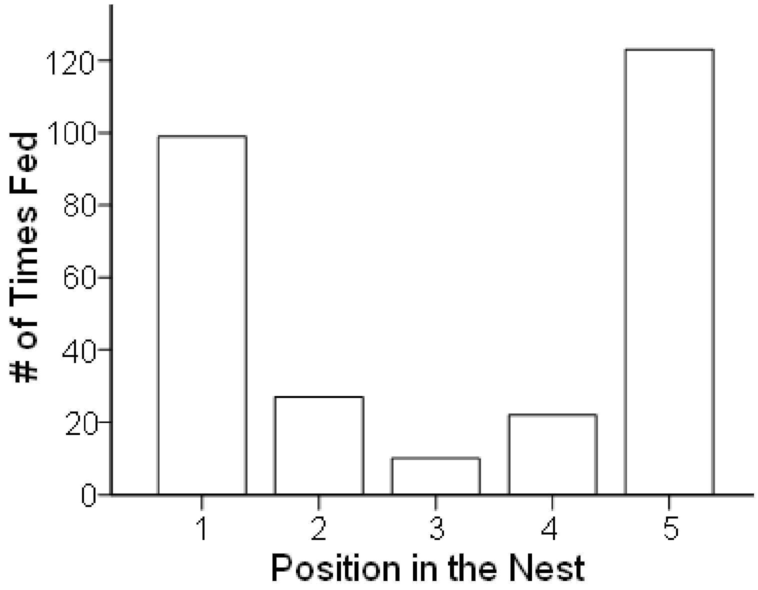

Figure 3. Positions in the nest occupied by nestlings when they received a feeding. Relative to nest entrance: position 1 was in the center of the nest, 2 was at the back of the nest, 3 was the left side, 4 was the right side, and 5 was below the entry hole.

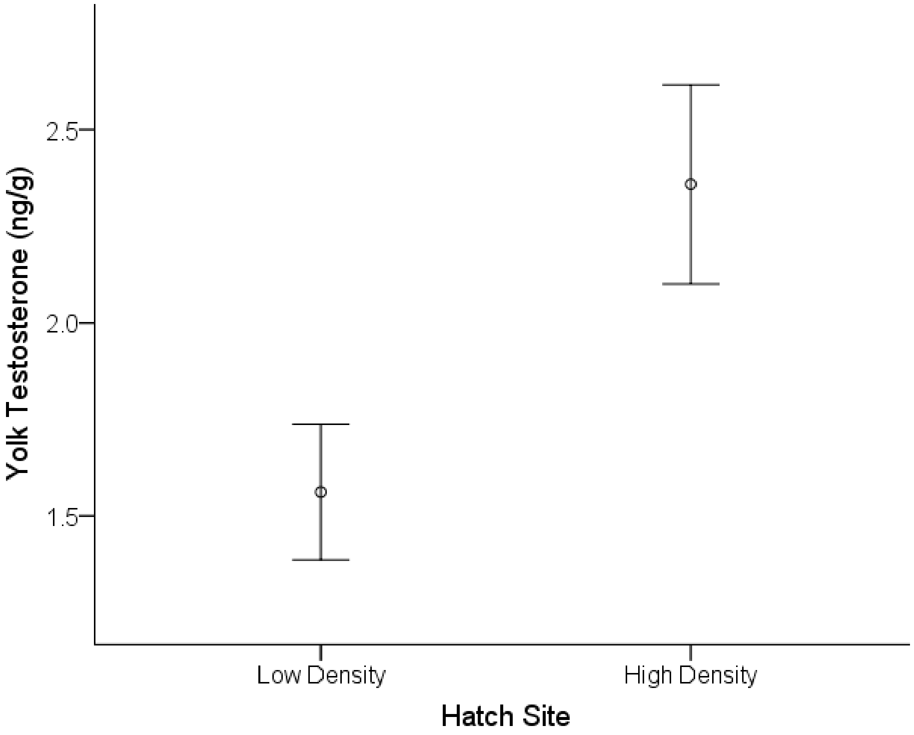

Figure 4. Yolk testosterone (ng/g) deposited in the 3rd egg of clutches from the high- and low-density sites. Error bars represent +/- 1 SE.

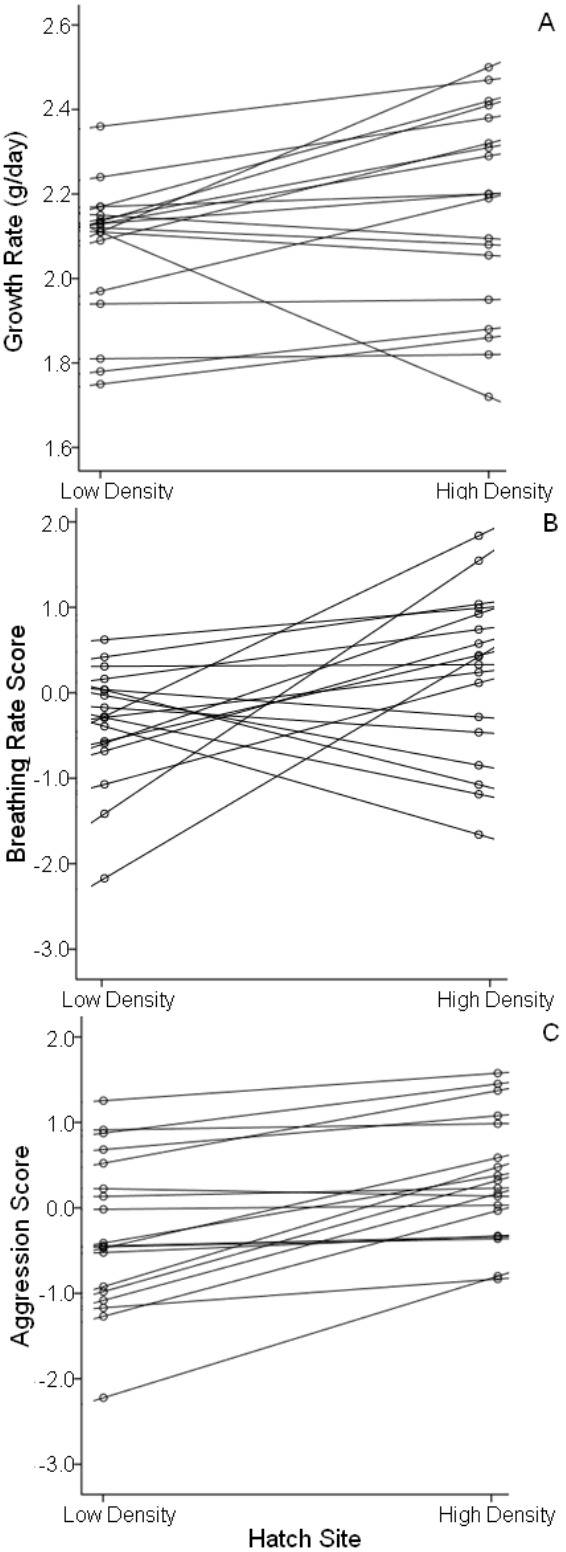

Figure 5. Comparison of fostermates hatched in high- and low-density sites to detect differences in A) growth rate, B) breathing rate (controlled for by air temperature) during handling stress, and C) aggression during feeding. Siblings raised in the same nest are averaged and represented by circles; lines connect fostermates.

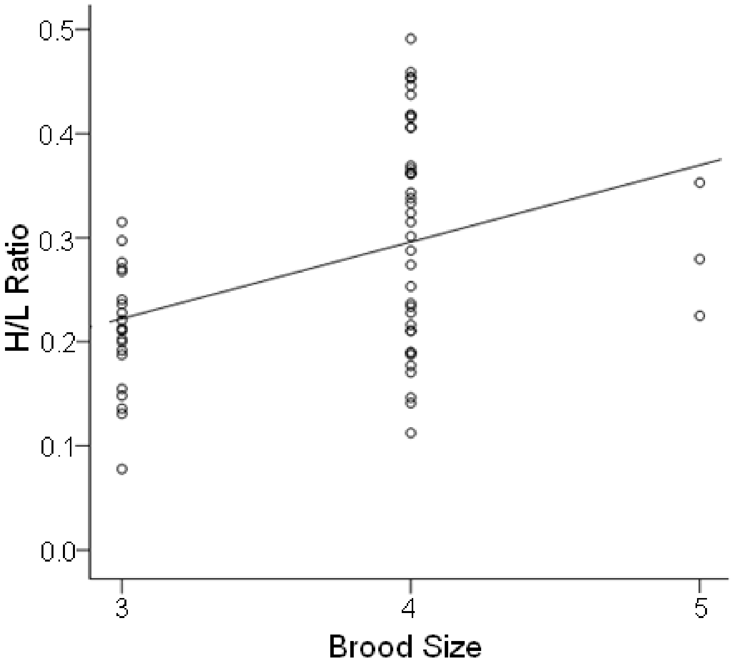

Figure 6. Influence of brood size ($F_{1,59} = 9.60$, $p = 0.003$) on H/L ratio of nestling tree swallows.

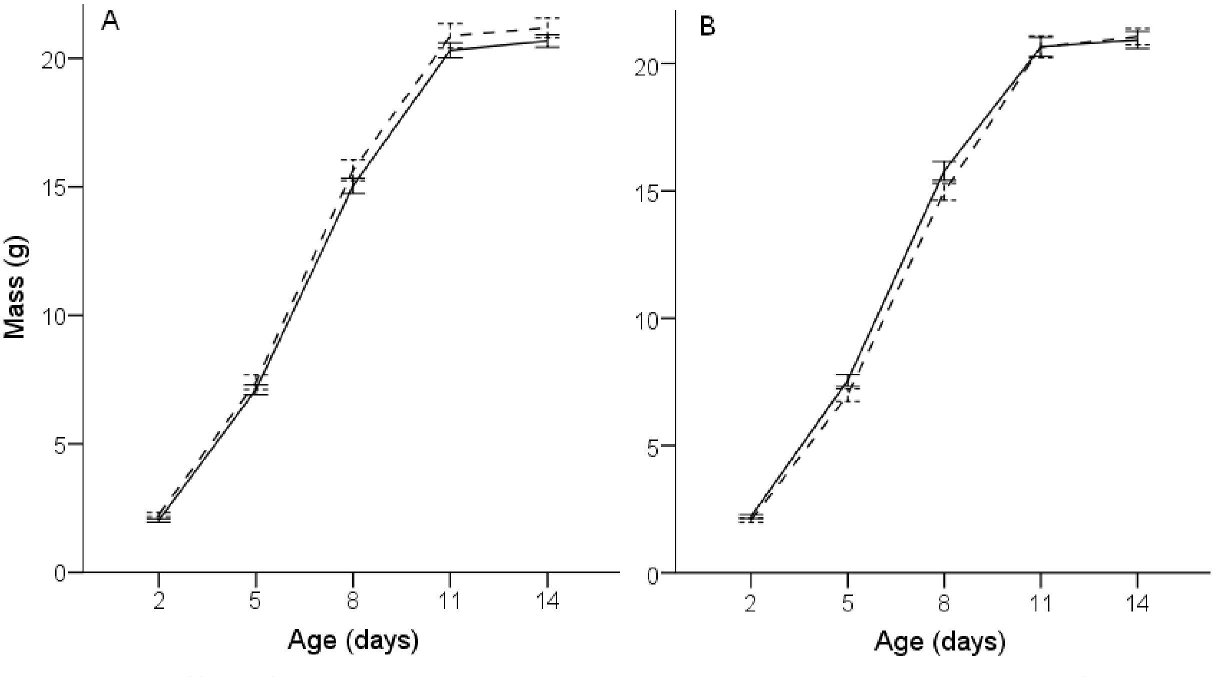

Figure 7. (A) Effect of yolk corticosterone ($F_{1,18} = 5.85$, $p = 0.03$) on growth prior to fledging in nestling tree swallows; below average yolk corticosterone is represented by dashed lines and bars and above average yolk corticosterone is represented by solid lines and bars. (B) Effect of hatch site density ($F_{1,186} = 5.28$, $p = 0.02$) on growth prior to fledging in nestling tree swallows; high-density hatch sites are represented by the solid line and bars and low-density hatch sites are represented by dashed line and bars. Bars represent +/- 1 SE.

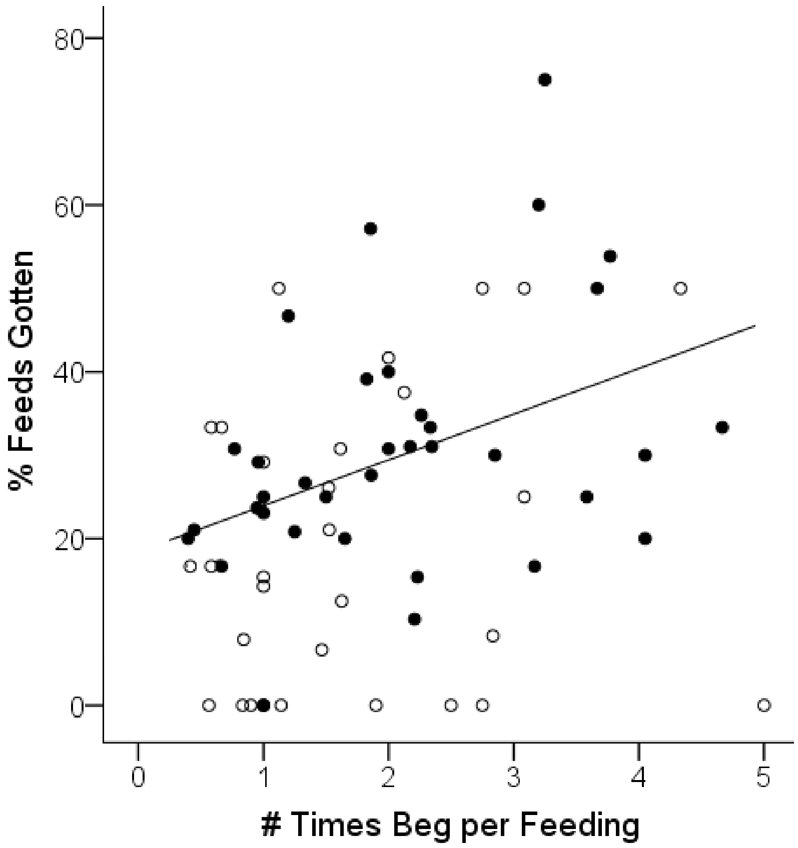

Figure 8. Relationship between begging rate and percent of feeds gotten by high aggression (closed circles and solid line; $r^2 = 0.16$, $F_{1,35} = 6.55$, $p = 0.02$) and low aggression (open circles; $r^2 = 0.03$, $F_{1,29} = 0.74$, $p = 0.40$) nestlings.

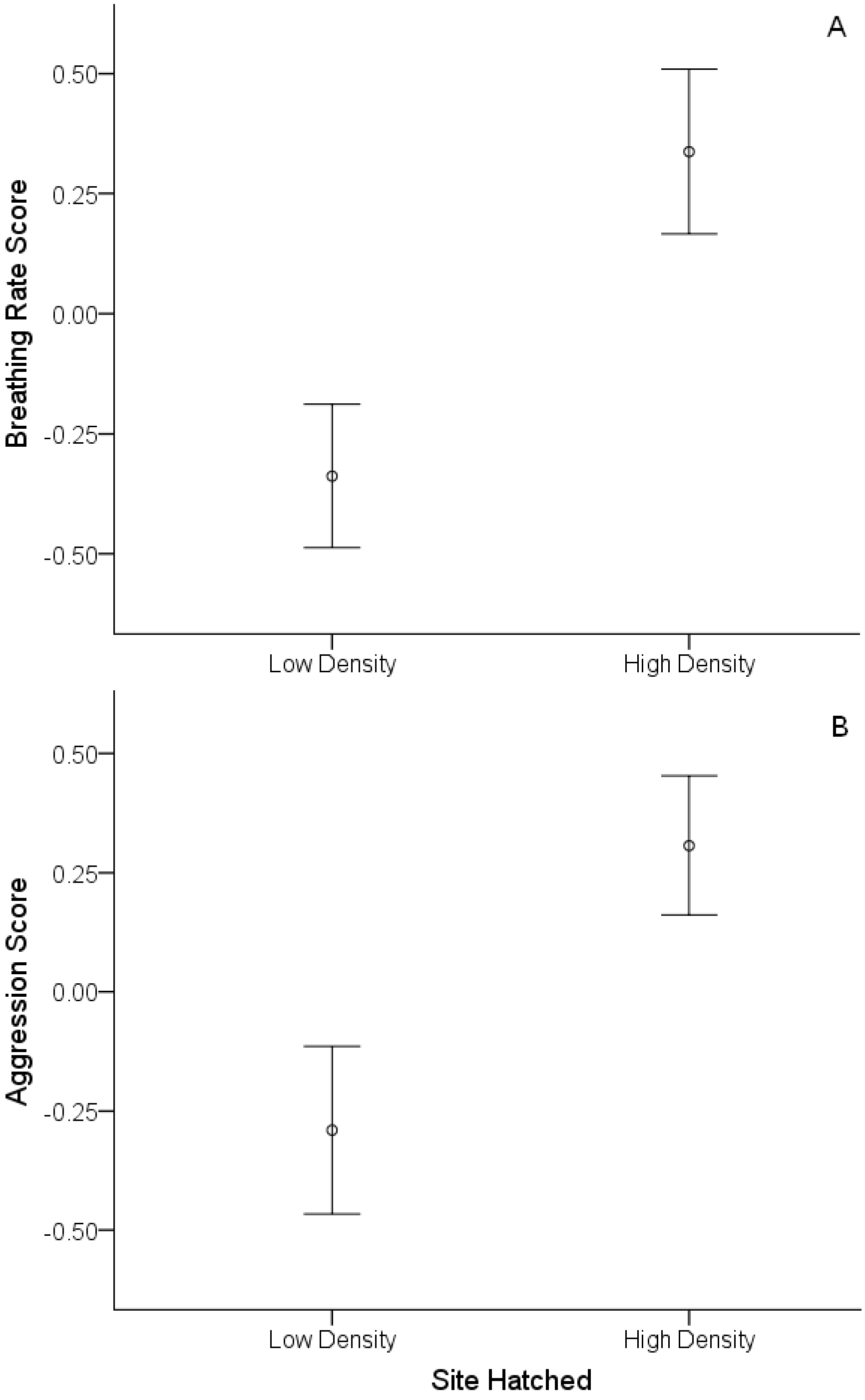

Figure 9. Effect of site hatched on mean behavior scores: A) Breathing rate during handling stress (controlled for by air temperature; $F_{1,51} = 5.93$, $p = 0.018$) and B) aggression during feeding ($F_{1,43} = 4.26$, $p = 0.045$). Error bars represent +/- 1 SE.

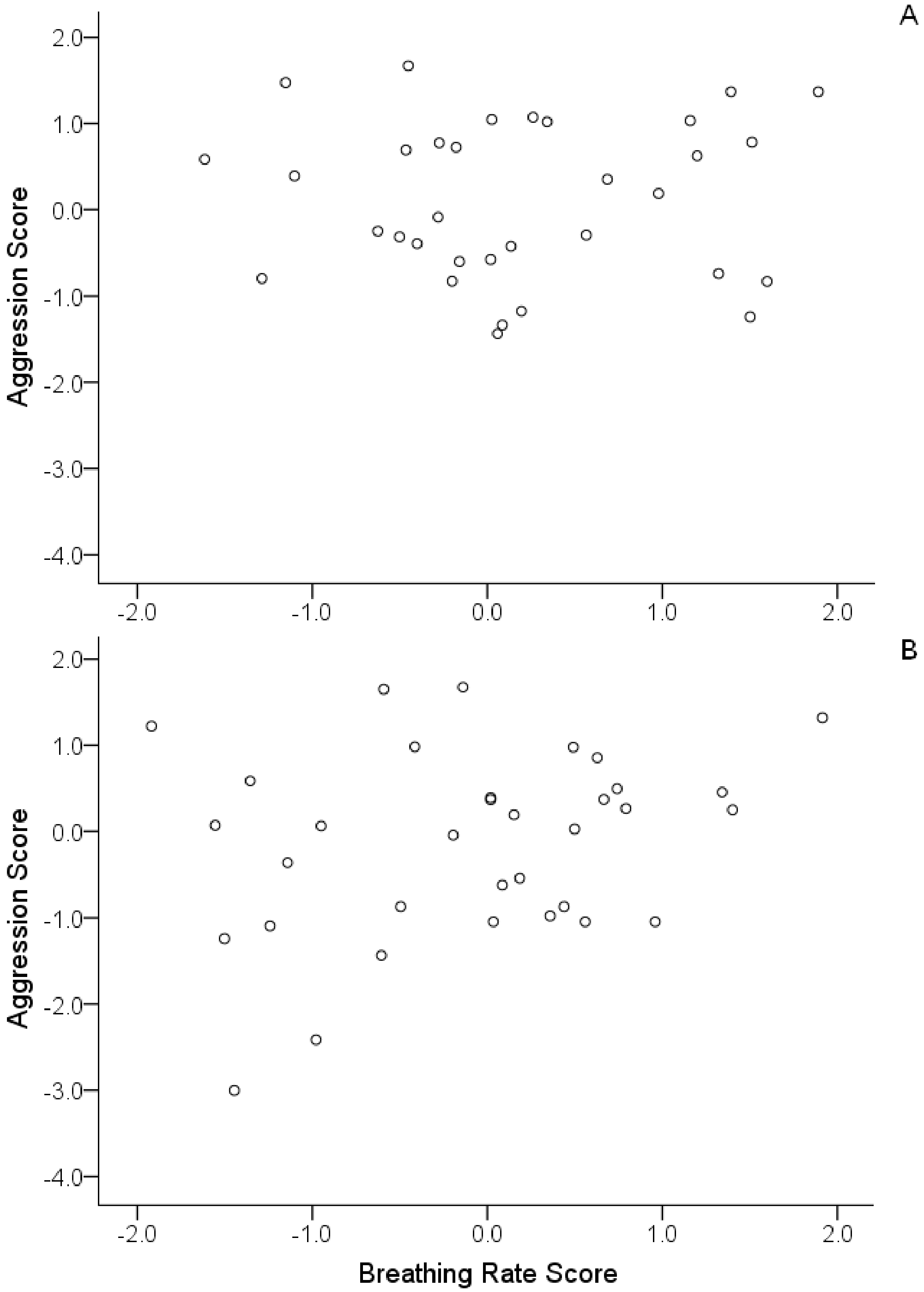

Figure 10. Correlation of aggression and breathing rate score for nestlings that were A) swapped (reared in an environment they were not hatched; $r = 0.02$, df = 33, $p = 0.93$) and B) not swapped (raised in the environment in which they were hatched; $r = 0.27$, df = 34, $p = 0.118$).

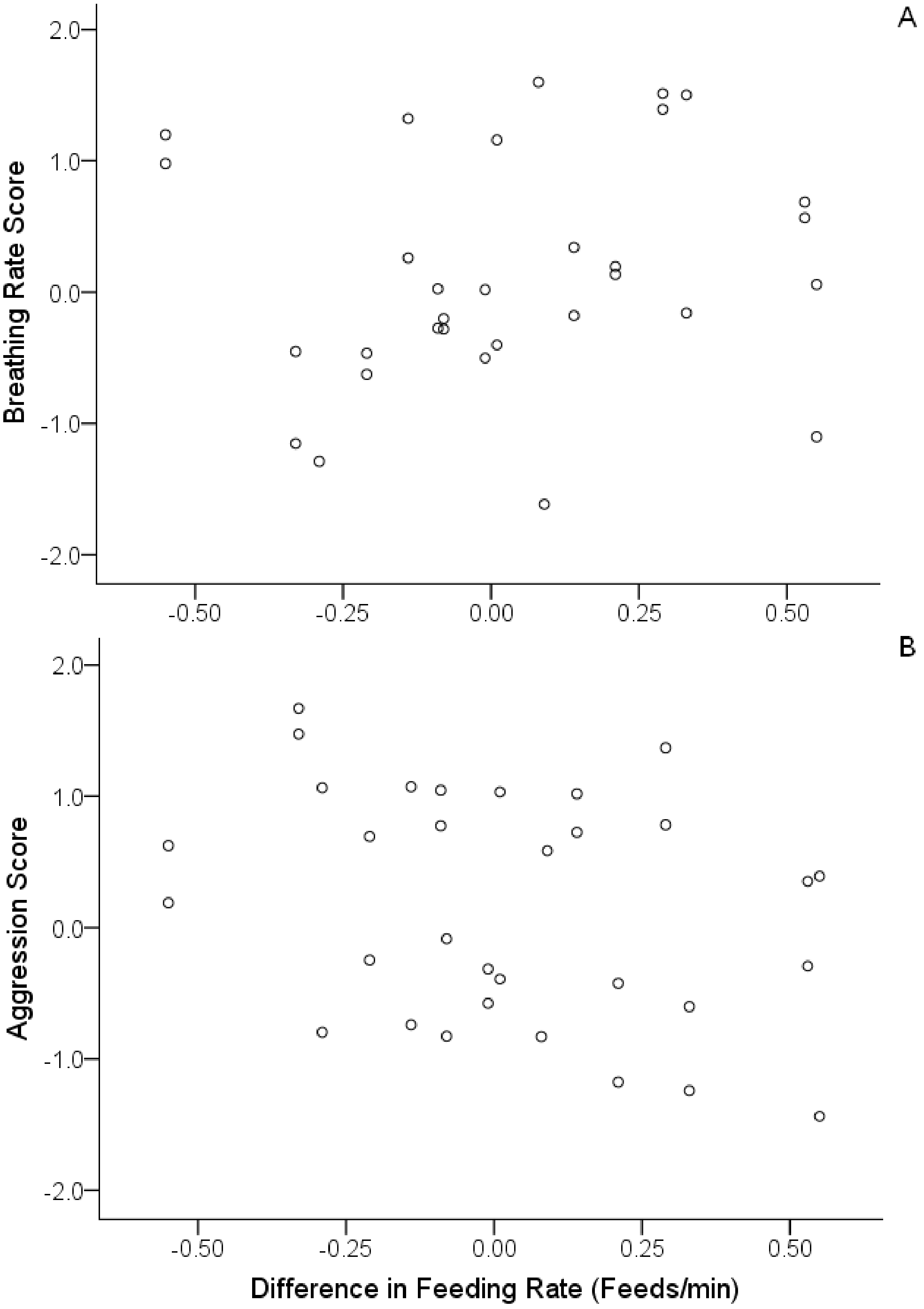

Figure 11. Correlation for difference in feeding rate (biological parents feeding rate minus foster parents feeding rate) on A) breathing rate (*r* = 0.14, df = 31, *p* = 0.47) and B) aggression score (*r* = -0.31, df = 32, *p* = 0.08) that were reared in an environment they were not hatched.

BIOGRAPHICAL INFORMATION

Alexandra Bea Bentz was born in Philadelphia, Pennsylvania, on April 20, 1988. Her parents are Catherine and Alvin Bentz and her sisters are Sarah Mayer and Jordan Bentz. She attended elementary school in Mount Lebanon, Pennsylvania. In 1994, her family was relocated to Mooresville, North Carolina, where Ms. Bentz attended middle school and high school. She graduated from Lake Norman High School in May 2006. In August 2006, Ms. Bentz enrolled in Appalachian State University in Boone, North Carolina, to study biology. She was awarded a Bachelor of Science degree in May 2010. In August 2010, Ms. Bentz accepted a graduate assistantship at Appalachian State University with Dr. Lynn Siefferman. She was awarded a Chancellor's Fellowship to fund her graduate studies for two years and received her Master of Science degree in biology in May 2012. In August 2012 she enrolled at Auburn University for her Doctor of Philosophy where she was awarded the Cellular and Molecular Biosciences Fellowship and the National Science Foundation Graduate Research Fellowship.