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## Effects of Corticosterone and Food Supplementation on Begging Behavior in Nestling Florida Scrub-Jays (*Aphelocoma Coerulescens*)

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EFFECTS OF CORTICOSTERONE AND FOOD SUPPLEMENTATION ON  
BEGGING BEHAVIOR IN NESTLING FLORIDA SCRUB-JAYS  
(*APHELOCOMA COERULESCENS*)

Emily Kate Elderbrock

A Thesis

Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
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## ABSTRACT

Elderbrock, Emily Kate. M.S. The University of Memphis. December 2012. Effects of corticosterone and food supplementation on begging behavior in Florida scrub-jay (*Aphelocoma coerulescens*) nestlings. Major Professor: Stephan J. Schoech.

In birds, altricial young depend upon adults for food and protection during the first few weeks of life. Begging is hypothesized to communicate an honest signal of a nestling's nutritional needs. I investigated two factors that may influence rate and duration of begging: 1) a nestling's corticosterone (CORT) levels, and 2) food availability. I examined the role of CORT in Florida scrub-jay nestlings through manipulation of CORT levels on days 8-11 post-hatch. I studied the role of food availability by indirectly supplementing all nestlings within a brood through their parents. I quantified nestling and adult behaviors using high definition video. CORT-treatment did not influence begging behaviors in nestlings in comparison to control nest mates. However, when the average of all nestlings within a CORT-treated nest was examined, I found an overall elevated begging rate compared to supplemented nestlings. I found minimal support for an effect of supplemental food on nestling and adult behaviors.

## PREFACE

My lab at the University of Memphis studies the physiology and behavior of Florida scrub-jays in the field at Archbold Biological Station in Florida. The primary focus of our work is currently the adrenal steroid hormone, corticosterone. This hormone is commonly referred to as a “stress hormone” because its secretion is markedly increased in response to a stressor. I pursued my own interests within these topics at Archbold Biological Station over two field seasons. The following is my research findings in my investigation of corticosterone and its relationship to nestling behavior. I am the primary author on both of these co-authored manuscripts. All chapters have been formatted to meet the style of the journal *Hormones and Behavior*.

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## CHAPTER 1: INTRODUCTION

The young of altricial bird species are helpless at hatching and depend on adults for protection and food as they develop in the nest. During this period, nestlings communicate their nutritional needs by begging. Begging, the act of raising the head and opening the beak (often accompanied by vocalization), is necessary for survival because in order to grow quickly, an individual must elicit feeding from adults. Despite the necessity of begging, there may be costs when begging reaches high levels. For example, the movement by nestlings while begging, as well as nestling calls may attract the attention of predators (McDonald et al., 2009). In addition, high levels of begging require increased energetic expenditure which may result in decreased immunocompetence and increased oxidative stress (Morena-Rueda, 2010; Morena-Rueda et al., 2012). Thus, an individual must balance its nutritional needs with the potential costs.

Recently, study of the hormonal regulation of begging has received increased interest. One of the primary hormones that mediates begging is corticosterone (CORT), the avian glucocorticoid. Evidence suggests that during periods of nutritional deficiency, CORT levels of nestlings increase (Kitaysky et al., 1999, 2001; Pravosudov and Kitaysky, 2006). This increase in CORT levels may in turn increase begging rates as the nutritionally stressed nestling attempts to gain additional food from adults (Kitaysky et al., 2003; Louiseau et al., 2008). This hormonal response to nutritional deficiency is in accordance with the known role of CORT in coping with a stressor.

CORT is constantly secreted at baseline levels, although baseline levels vary with circadian and circannual rhythms, and is essential for daily maintenance (Reeder and Kramer, 2005). However, when homeostasis is disrupted or when an individual perceives a stressor, a dual-phased stress response occurs. First, within seconds, epinephrine and



norepinephrine (adrenalin and noradrenalin) are released. If the stressor persists, the hypothalamus secretes corticotrophin-releasing hormone (CRH) that then stimulates the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH) which, in turn signals the adrenal cortex to release CORT (Sapolsky, 2000). This pathway is referred to as the hypothalamic-pituitary-adrenal (HPA) axis and its activation results in increased CORT secretion within a few minutes (see Romero and Reed, 2005). Together, these two phases initiate a number of actions that facilitate the appropriate response to the stressor. These include increased glucose production and blood flow to necessary tissues (i.e., skeletal muscles for flight) (Wingfield, 2005; Romero and Butler, 2007). Most research into the stress response has been conducted on adults; however, nestlings of several species also exhibit a stress response (Sims and Holberton, 2000; Wada et al., 2007; Rensel et al., 2010). If nutritional deficiency initiates a stress response in a nestling, then the corresponding increase in CORT may alter begging behavior to ensure the individual receives more food from its parents.

I investigated two factors that may impact begging rates: corticosterone and food availability. In the first study, I manipulated CORT levels in Florida scrub-jay (*Aphelocoma coerulescens*) nestlings by feeding them CORT-injected waxworms. I hypothesized that CORT would influence the begging rates and growth of treatment nestlings. Specifically, I predicted that CORT treated nestlings would express increased begging rates and decreased somatic growth.

In my second study, I investigated the begging behavior and plasma CORT levels of nestlings in a subset of nests that received supplemental food. After eggs hatched, radio-frequency identification (RFID)-enabled *SmartFeeders* provided ad libitum meal

worms to an adult breeder. Behavioral observations found that around 50% of the worms taken from the feeder by a breeder were fed to that individual's nestlings (Small and Schoech, unpublished data). I hypothesized that supplemental feeding would influence nestling begging rates and plasma CORT levels. I predicated that nestlings that received supplemental food would have decreased begging rates and lower plasma CORT levels than non-supplemented nestlings.

For references see Chapters 2 and 3.

## CHAPTER 2

### Exogenous corticosterone does not influence begging behavior or growth in nestling Florida scrub-jays (*Aphelocoma coerulescens*)

#### Introduction

Many organisms are born helpless and rely on their parents to provide them with their basic needs in order to grow and survive to nutritional independence. In birds, young of altricial species remain in the nest as they develop and are dependent upon adults to provide food and protection. During this period, nestlings communicate their needs by begging, which is thought to be an honest signal of an individual's nutritional needs (Kilner and Johnstone, 1997; Royle et al., 2002). When a nestling requires food, it will beg to elicit feeding from its parents. The hormonal regulation of begging has been of interest recently and the avian glucocorticoid, corticosterone (CORT), has been identified as an important regulator of this behavior (Smiseth et al., 2011).

CORT is commonly thought of as a “stress hormone” because it is secreted in response to stressful stimuli and subsequently stimulates responses that allow the individual to cope with the stressor. For example, it allows for increased glucose production and blood flow to the necessary tissues (e.g., skeletal muscles), both of which support the fight-or-flight response (Wingfield, 2005; Romero and Butler, 2007). In addition to being released in response to a stressor, CORT is also essential for normal development and many aspects of day-to-day life. A “baseline” CORT level is the amount of hormone that is secreted at low-levels which vary with circadian and circannual rhythms (Sapolsky, 2000; Reeder and Kramer, 2005). CORT secretion is essential, both for routine physiological function, as well as by increasing an individual's chance of survival during a potentially harmful event (Pravosudov, 2003; Breuner, 2008).

However, mounting a stress response is not without costs, as doing so is energetically expensive and chronic CORT secretion can have multiple negative effects on an organism regardless of its age (Sapolsky, 2000; Reeder and Kramer, 2005; Wingfield, 2005).

In the first few weeks of life, altricial nestlings undergo rapid somatic growth along with growth and development of multiple physiological systems, such as the hypothalamic-pituitary-adrenal (HPA) axis. Altricial nestlings typically have a functional, although dampened, stress response prior to fledging (Sims and Holberton, 2000; Wada et al., 2007; Rensel et al., 2010). Rensel et al. (2010) tested day 11 post-hatch nestlings of our study species, the Florida scrub-jay (*Aphelocoma coerulescens*, FSJ), and found they responded to handling stress as the initial rate (i.e., at 0-5 min) of CORT increase did not differ from that of adults, although their mean maximum level was less than half that of adults. This inability to mount a full response could reflect incomplete HPA axis competency or a protective mechanism against negative effects of CORT (Rensel et al., 2010). Young individuals are especially vulnerable to CORT during early development when increased levels can impact central nervous system (CNS) development, an effect that can have long-term lasting effects, including shaping an individual's behavioral and physiological phenotype (Kitaysky et al., 2006; Spencer and Verhulst, 2008; Wada and Breuner, 2008; Schoech et al., 2009). Exposure to CORT during development may program an individual for the environmental conditions that it will experience once it has left the nest (see Schoech et al., 2011 for review). For example, if a nestling experiences harsh conditions in the nest (i.e., food deprivation due to poor environmental conditions), then it may express a phenotype that is ultimately best suited to cope with suboptimal

conditions after fledging. Schoech et al. (2009) found that FSJ nestlings with higher baseline CORT levels on day 11 post-hatch, expressed a more “fearful” behavioral phenotype (i.e., an increased latency to approach a novel object and more pronounced startle responses) at approximately 7 months of age when compared to nestlings with lower baseline CORT levels. Considerable evidence across taxa indicates that CORT exposure during development can shape the adult phenotype (reviewed in Schoech et al., 2011).

In addition to long-term effects, CORT may have short-term effects upon nestlings while in the nest. For instance, if a nestling is food deprived, increased CORT levels may induce increased begging to ensure that the individual receives more food from its parents (Kitaysky et al., 1999). Study of the hormonal regulation of begging is currently limited to a few studies, and these have differed in their findings (reviewed by Smiseth et al., 2011). The effects of CORT on begging have been studied in individuals by manipulating CORT levels during development. Exogenous CORT caused black-legged kittiwakes (*Rissa tridactyla*) and house sparrow (*Passer domesticus*) nestlings to increase begging rates (Kitaysky et al., 2001, 2003; Louiseau et al., 2008). In contrast, Wada and Breuner (2008) found CORT increased the latency to beg in white-crowned sparrows (*Zonotrichia leucophrys nuttallii*) and Vallarino et al. (2006) found exogenous CORT did not affect begging rates in blue-footed boobies (*Sula nebouxii*). In addition to having an effect on begging, exogenous CORT also decreased rates of somatic development in nestlings (Spencer and Verhulst, 2007; Wada and Breuner, 2008). Such effects would likely put individuals at a disadvantage because those that grow the fastest and become the largest within the nest invariably out-compete their nestmates and

subsequently have the best chance of survival post fledging (Magrath, 1991, Kersten and Brenninkmeijer, 1995).

We manipulated CORT levels of FSJ nestlings by feeding the nestlings CORT-injected wax worms (*Galleria mellonella*) for a period of 3.5 days. CORT levels were increased to physiologically relevant stress-induced levels found previously in FSJs (see Rensel et al., 2010). The primary aim of this study was to investigate the short-term effects of CORT on begging behavior and somatic growth. We predicted that CORT-treated nestlings would express increased begging rates and decreased somatic growth when compared to controls.

## **Methods**

### *Study System*

This study was performed during the field season of 2012 on a well-studied, free-living population of Florida scrub-jays at Archbold Biological Station in south-central Florida (27°10'N, 81°21'W). FSJs are non-migratory, socially and genetically monogamous, and live in family groups that average three jays (Woolfenden and Fitzpatrick, 1984; Quinn et al., 1999; Townsend et al., 2011). Each bird in the population is uniquely color-banded and monitored from hatching until death. FSJs begin nesting in early March with a mean clutch size of three eggs and incubation and nestling periods that both average 18 days. FSJ nestlings are altricial and rely on parents and non-breeding “helpers” for protection and nourishment while in the nest and until nutritional independence at approximately 70 days post-hatch (Woolfenden and Fitzpatrick, 1984). Approximately half of the breeding pairs in our population have from 1-6 helpers with a mean group size of 3.

All FSJ nests were located during the nest building stage and monitored until the nest was abandoned, depredated, or successfully fledged young. Frequent nest checks on the predicted day of hatching allowed us to record hatch order, determine mass at hatching, and uniquely mark a toenail of each nestling. Toenails were repainted on days 3, 5, and 8 post-hatch, thereby allowing individuals to be monitored throughout the nesting period. On days 5 and 8 post-hatch, nestlings were reweighed and the tarsus was measured. On day 11 post-hatch, each nestling was banded with a single plastic color band and an U.S. Geological Survey aluminum band, and we also measured individuals' tarsus, 7<sup>th</sup> primary feather, and body mass. Mass taken on hatch day, day 5, day 8, and day 11 were used to calculate the growth rate of each nestling. Growth rate was calculated as the mean daily change in mass.

#### *Corticosterone Treatment*

The role of CORT was examined by feeding CORT-injected wax worms to treatment individuals during the nesting period. Only the first two hatched nestlings from each nest were treated: one received a CORT-injected worm (treatment) and the second received a worm injected with vehicle only (control). Treatment and control assignment were alternated between the first and second hatched nestlings in subsequent nests. This method allowed us to pair treatment and control individuals within a nest and thereby control for nest environment. Other nestlings in the nest received no worm (non-treated). Crystalline CORT (0.8 mg, Sigma C2505) was dissolved in 1 ml of peanut oil and preparation of individual doses were accomplished by injecting 20  $\mu$ l (16  $\mu$ g of CORT) of solution into a wax worm on day 8 and, to account for the increase in body mass, 25  $\mu$ l on days 9-11 (modified from Wada and Breuner, 2008, see also Schoech et al., 2007).

Control doses were wax worms that had been injected with 20  $\mu$ l or 25  $\mu$ l of peanut oil. A preliminary study in 2011 found that nestling CORT levels were successfully increased through the feeding of the CORT-injected worms (Elderbrock, unpublished data).

Nestlings were fed either a control or hormone-injected wax worm twice daily (between 0700-0900 and 1630-1830) from days 8 – 10 post-hatch, and once in the morning on day 11. On day 11, all nestlings were removed from the nest 20 minutes after dosage to collect a blood sample to assess the efficacy of treatment and to determine if CORT levels were near target levels based on the endogenous levels reported by Rensel et al. (2010).

#### *Blood Sampling and Corticosterone Assay*

Blood samples from all nestlings were collected on day 11 post-hatch. To ensure that the sample would reflect baseline CORT levels, we used multiple field researchers to remove all nestlings from the nest simultaneously (time 0). A small blood sample (~100  $\mu$ l) was taken from the brachial vein of each individual within three minutes of removal from the nest to determine baseline plasma CORT levels (Romero and Reed, 2005). Nestlings were held in the hand and at 10 minutes post nest removal we took a second blood sample (~100  $\mu$ l) to determine a stress-induced CORT level. Blood samples were placed on ice until return to the lab (within an hour of blood sampling) where they were centrifuged for 5-7 minutes at 12000 rpm. The plasma was drawn off and frozen at -20°C until analysis at the University of Memphis.

Samples were assayed using the Cayman 500655 Corticosterone Enzyme-Linked Immunosorbent Assay (ELISA; Cayman Chemicals, Ann Arbor, MI) following the recommended protocol. All samples were run on the same day. In all instances, baseline



and stress-induced samples from all individuals within a nest were run on the same plate. Baseline samples were diluted 1:20 in EIA buffer and stress-induced samples were diluted 1:100 prior to assaying. These dilutions were used to assure that binding values were within the linear portion of the sigmoidal standard curve (i.e., between 15 and 65% bound). CORT standards of known concentration were included on each plate (intra-assay CVs  $\leq 3\%$ ; inter-assay CV  $\leq 5\%$ ).

### *Nest Monitoring*

We used *TowerCams*, a high definition camera (JVC HD Everio, GZ-HM440) mounted atop a 1.9 meter pole that can extend up to 3.2 meters, to monitor behavior at the nest. Most FSJ nests are 1-2 meters off the ground (Woolfenden and Fitzpatrick, 1984) and the vantage point of the cameras allowed us to monitor individual nestling and adult behavior from a distance that did not disturb the adult jays. A preliminary study in 2011 found that adult behaviors were not altered by the presence of a camera (Elderbrock, unpublished data). Recording began between 7:30 and 11:00 on days 5, 8, 10, and 13 post-hatch. The camera would be set-up and turned on and the individual nestlings and their position within the nest were then determined by their toe mark. Because I knew the position of each individual nestling at the beginning of recording, I could assess begging behavior and parental feeding for each individual for the duration of the video. The nests were filmed for 90-120 minutes. The variation in recording duration was due to limited camera availability when videoing at multiple nests in a single morning, and the fact that some sessions were shortened by rain. The lack of standardization in recording duration has been compensated for in the analyses by expressing variables as rates per hour.

### *Video Analysis*

The videos were used to monitor both nestling and adult behavior. For each recording we measured the number of begs and duration of begging bouts, as well as the number of times each individual nestling was fed. A “beg” was counted every time a nestling raised its head and opened its beak. The “beg duration” was the time a nestling kept its beak open while craning its neck in a characteristic begging posture for each beg. Each beg received a minimum duration of one second, although most were far longer. A “feed” was counted when an adult put food into an individual’s open beak and the individual swallowed the food. Each variable was converted to a rate per hour per individual for statistical analyses.

### *Statistical Analysis*

To compare behavioral measurements between CORT-treated and control nestlings, we used a two-way repeated measures ANOVA across days. In cases where the effect of day was significant, we used a Holm-Sidak post hoc test to determine differences between days. Baseline and stress-induced CORT levels were log transformed to meet assumptions of normality. We used a two-way repeated measures ANOVA to compare baseline and stress-induced CORT levels between treatments. One-way ANOVAs were used to compare growth rate and day 11 morphometrics (mass, length of tarsus and 7<sup>th</sup> primary feather) of the treatment and control individuals. SigmaPlot 12.0 was used for statistical analysis.

## **Results**

There was no effect of treatment on baseline or stress-induced CORT levels (Fig. 1,  $F_{(1,27)} = 1.11$ ,  $P = 0.31$ ), but there was an effect of time ( $F_{(1,27)} = 22.65$ ,  $P < 0.0001$ ). In

addition, there was a significant interaction of CORT treatment and the two time-points ( $F_{(1,27)} = 6.3, P = 0.027$ ). Treatment nestlings had higher baseline CORT levels compared to control nestlings ( $P = 0.02$ ) but stress-induced CORT levels were not different ( $P = 0.34$ ). CORT levels did not change from baseline to stress-induced in the treatment nestlings ( $P = 0.14$ ), but did change in control nestlings ( $P < 0.001$ ).

Begging rates did not differ between treatment and control birds (Fig. 2,  $F_{(1,53)} = 0.32, P = 0.58$ ). A day effect was found ( $F_{(1,53)} = 9.36, P = 0.0035$ ) with a greater begging rate on day 13 than the other days (day 5:  $P < 0.001$ ; day 8:  $P = 0.002$ , day 10:  $P = 0.007$ ). There were no other differences among days. There was no interaction between treatment and day ( $F_{(3,53)} = 0.76, P = 0.52$ ).

Begging duration did not differ between treatment and control birds (Fig. 3,  $F_{(1,53)} = 0.39, P = 0.55$ ) or among days ( $F_{(3,53)} = 2.09, P = 0.12$ ). The interaction of treatment and day also was not significant ( $F_{(3,53)} = 0.24, P = 0.87$ ).

The number of feeds by parents did not differ by treatment ( $F_{(1,53)} = 0.57, P = 0.46$ ) or across the four days of recording ( $F_{(1,53)} = 1.49, P = 0.24$ ). Neither was there an interaction between day and treatment on the number of feeds ( $F_{(3,53)} = 1.29, P = 0.29$ ).

There was no difference in growth rate ( $F_{(1,13)} = 1.07, P = 0.32$ ). Nor were there any treatment effects on any of the day 11 morphometrics: mass ( $F_{(1,13)} = 0.28, P = 0.61$ ), tarsus ( $F_{(1,13)} = 0.075, P = 0.79$ ), and 7<sup>th</sup> primary feather ( $F_{(1,13)} = 0.39, P = 0.54$ ).

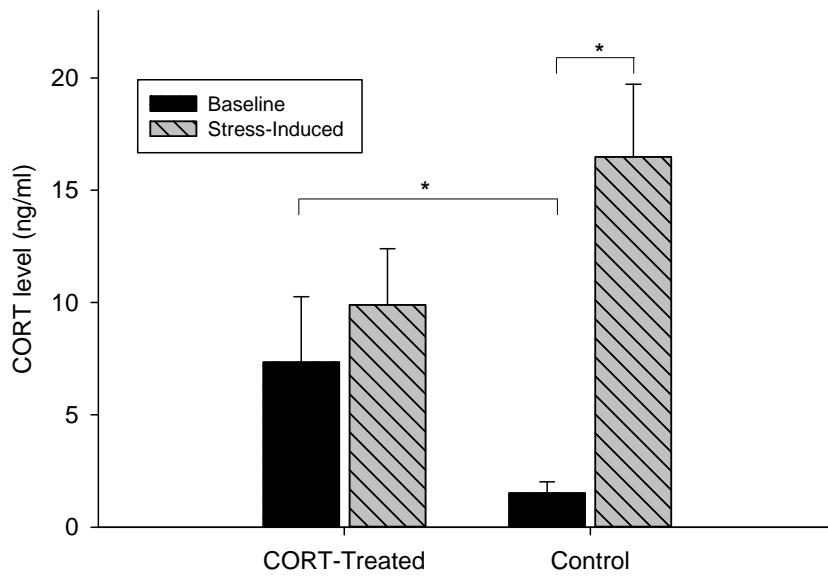


Figure 1. Baseline and stress-induced CORT levels of treatment (n = 7) and control (n = 7) nestlings. Error bars indicate standard error. Symbols indicate significant difference.

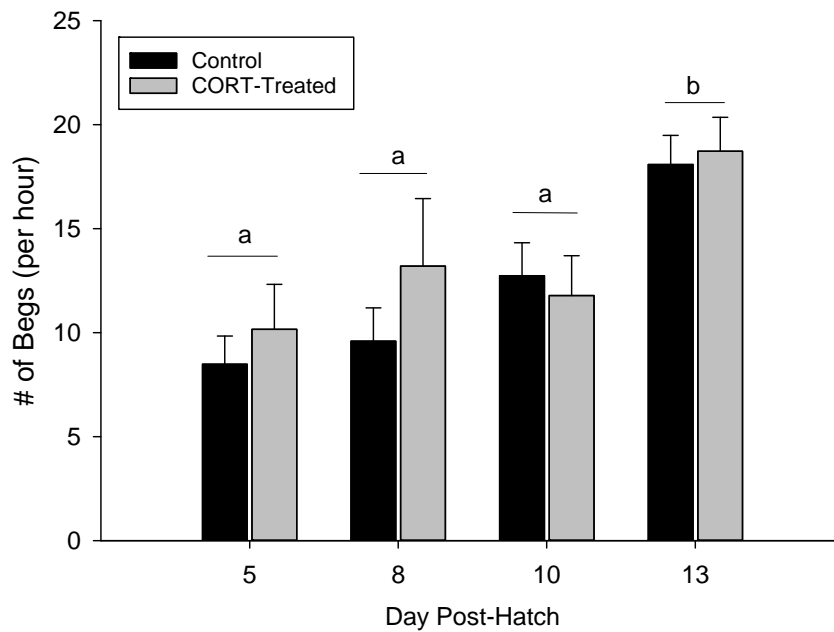


Figure 2. The number of begs per hour between CORT-treated and control individuals across days. Error bars indicate standard error. Letters indicate significant difference among days (all treatments combined).

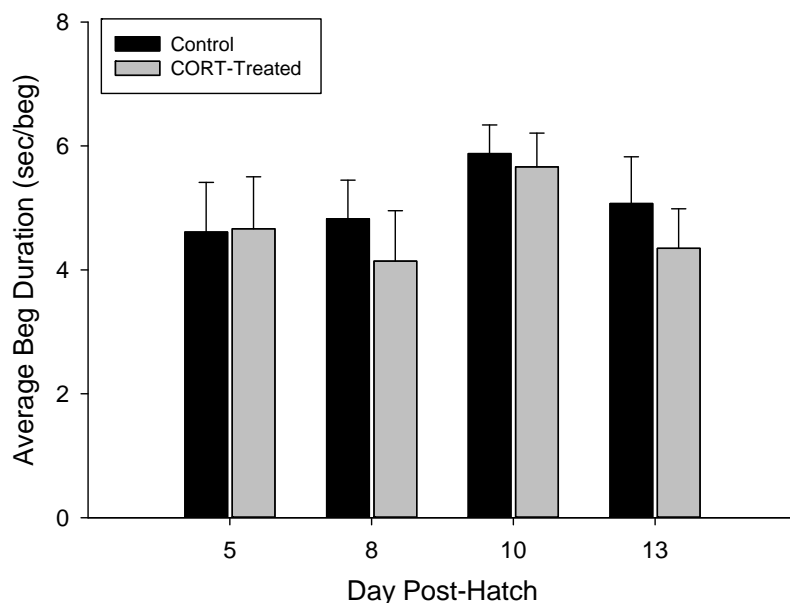


Figure 3. Average time per beg (seconds/hour) between CORT-treated and control individuals across days (all treatments combined). Error bars indicate standard error.

## Discussion

We investigated the effects of exogenous CORT on begging behavior and somatic growth in FSJ nestlings. We predicted that begging rate and begging duration would be increased in CORT-treated individuals; however, no behavioral differences were found between the treatment and control birds within the same nest. In addition, growth rate and other measurements of somatic growth were not affected by the treatment.

Baseline CORT levels were increased in treatment birds, confirming the efficacy of the treatment. However, after 10 minutes of handling, while CORT levels of controls increased markedly, CORT-treated nestlings exhibited little change (Fig. 1). This overall lack of a treatment-effect in stress-induced levels is due to the relatively small increase from baseline exhibited by CORT-treated individuals. Baseline CORT samples were

taken 20 minutes post-dosage and the stress-induced sample was taken 10 minutes post-nest removal or 30 minutes post-dosage. By this point the CORT-treated individuals had likely reached peak levels as a result of the exogenous CORT. The damped CORT-response to handling may be attributable to activation of negative feedback systems that minimized the endogenous CORT-response to the stressor. Alternatively, the reduced CORT-response in the treated nestlings might be due to the longer-term effects of the multiple days of CORT treatment.

We hypothesized that increased CORT would have a short-term activational effect on begging as has been found in previous studies (Loiseau et al., 2008; Wada and Breuner, 2008). We recorded behavior at four points during the nesting period, once on day 5 before treatment, on days 8 and 10 immediately following dosage, and a final time on day 13 two days after the last CORT dose. Despite an overall increase in begging rate and duration across observation days, we found no differences in begging rate, begging duration or parental feeding rate between treatment and control birds on any of the four days (Fig. 2 and 3). The lack of difference could be a result of the limited sample size (a result of a poor breeding season for all FSJs). Alternatively, the failure to detect differences could be a result of the procedure used to analyze the video recording. The entire duration of the recording (90-120 minutes) was used to extract the above measures, but perhaps limiting data collection to the first 30 minutes would be more appropriate. In a study with adult FSJs that used a similar protocol to elevate CORT levels, Schoech et al. (2007) found that CORT levels had returned to baseline 1 hour post-treatment. It may be that CORT has an effect upon behaviors only when elevated (i.e., within 20-30

minutes post-dosage). Future analysis will consider this short time period immediately after dosage.

We found no difference in growth rate or any of the other measures of somatic growth between groups. The CORT doses used may not have elevated CORT levels high enough or sustained this elevation long enough to result in decreased somatic growth. Muller et al. (2009) found a reduction in growth after a 2-3 day CORT-treatment in free-living Eurasian kestrels (*Falco tinnunculus*); however, nestlings received CORT implants that likely delivered a relatively constant dose of exogenous CORT. In addition, Wada and Breuner (2008) found a negative relationship between growth and CORT after only 24 hours of CORT treatment (CORT was delivered with a dermal patch). It is possible that our protocol of twice-a-day dosing is sufficient to briefly increase CORT, but that nestlings clear the CORT relatively quickly with no observable effect on growth and behavior.

Conflicting results in experiments employing transient CORT elevation suggest that the effects of CORT treatment may be age and dose dependent. For example, Loiseau et al. (2008) injected CORT once daily in house sparrows for a period of 5 days starting 5 days post-hatch and found an increase in begging rate. However, Wada and Breuner (2008) used three different age groups of nestling white-crowned sparrows and determined that feeding CORT increased the latency to beg, but in mid-staged nestlings only. Interestingly, they found growth was most strongly affected in the youngest nestlings. Given that altricial young may have little to no stress responsiveness immediately post-hatch and that responsiveness develops as they age (Sims and Holberton, 2000; Blas et al., 2006; Rensel et al., 2010), it is likely that a nestling's

vulnerability to the effects of CORT vary with the different stages of development. We chose to begin our CORT treatment on day 8, which is the day when a nestling's eyes typically begin to open, and stopped on day 11. Previous work in FSJs revealed a significant correlation between a nestling's day 11 baseline CORT level and their adult behavioral phenotype (Schoech et al., 2009), suggesting that by day 11, a nestling's CORT response phenotype has already been established. We were interested in the period from day 8 to day 11 in this study, but it is possible that FSJs are vulnerable to the effects of CORT prior to day 8.

Alternatively, we must address the possibility that FSJ nestlings are buffered from the negative effects of CORT during development. In this case, despite increased CORT levels, no behavioral or somatic differences are identified because the individual has measures in place to cope with the elevated levels of CORT to prevent adverse effects at such an early stage. One such buffer mechanism could be at the level of CORT receptors. For example, if CORT receptor levels remain relatively high in FSJ nestlings up until day 11, this could result in increased negative feedback which may prevent a behavioral response (Wada and Breuner, 2010). Nothing is known about CORT receptor levels in developing FSJs, but at this time we cannot rule out their involvement. Unfortunately, the threatened species status of the FSJ will prevent evaluation of receptor populations.

## **Conclusion**

Nestlings may be exposed to increased CORT levels as a result of poor environmental conditions and such an increase may be beneficial by allowing for a short-term increase in begging during a period of low food availability. In this study we found no difference in begging rates, begging duration, or parental feeding rates between treated



and control birds. In addition, CORT had no effect on somatic growth. Long-term studies will be carried out to consider long-term effects of CORT on the physiological and behavioral phenotypes of FSJs. However, this study suggests that increased CORT during development is not detrimental to an individual over the short-term.

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## CHAPTER THREE

### Effects of food supplementation on begging and corticosterone levels in nestling

#### Florida scrub-jays (*Aphelocoma coerulescens*)

##### Introduction

Begging by altricial nestling birds is believed to be an honest signal of their nutritional needs (Kilner and Johnstone, 1997; Royle et al., 2002). If a nestling requires food, it will beg to inform and elicit feeding from its parents. A nestling is most vulnerable to predation in the early stages of development (Schaub et al., 1992), thus a nestling needs to grow and develop quickly. Begging as often as possible may ensure it receives sufficient food; however, begging has costs which may limit the amount an individual begs. One potential cost of begging is increased predation risk (Leech and Leonard, 1997). Begging is often accompanied by vocalizations and McDonald et al. (2009) found that increased sound coming from the nest increased predation in bell miners (*Manorina melanophrys*). Increased begging may also have physiological costs, such as decreased immunocompetence (Morena-Rueda, 2010) and increased oxidative stress (Morena-Rueda et al., 2012). These factors may result in decreases in health and growth rate, both of which can reduce survival over the short- and long-term (i.e., both pre- and post-fledging). Thus, an individual's begging rate likely represents a compromise between positive and negative selective pressures on begging, such that an individual begs at a rate that is sufficient to elicit adequate parental care, while minimizing costs.

In addition to the costs of begging, the interests of an individual offspring and its parents may diverge. While the offspring is only mindful of its own needs, a parent must balance its own needs with those of its young, a consideration that can be further

complicated with multiple offspring ('parent-offspring conflict', Trivers, 1974). In large broods, siblings compete with each other to receive adequate nutrients from often limited resources (Godfray and Johnstone, 2000). Food availability may also influence begging and feeding rates when environmental conditions are good. For example, Quillfeldt and Masello (2004) found that Cory's shearwater (*Calonectris diomedea*) nestlings that received supplemental food were satiated and ceased to beg and the parents decreased their nest attendance. If a nestling does not need food, it saves energy and avoids potential costs by not begging; similarly, an adult saves energy by not supplying unneeded food.

When resources are limited and a nestling is nutritionally deficient, it may result in increased secretion of the avian glucocorticoid, corticosterone (CORT) (Kitaysky et al., 1999, 2001; Pravosudov and Kitaysky, 2006). CORT secretion at baseline levels are essential for developmental processes in the young and for maintenance of physiological systems in adults. CORT secreted in response to a stressor facilitates adaptive physiological and behavioral responses that help the individual to cope with the stressor (Sapolsky, 2000; Reeder and Kramer, 2005). However, CORT exposure during development may have short- and long-term implications in shaping an individual's behavioral and physiological phenotypes, both of which may ultimately impact its survival (see Chapter 1 and Schoech et al., 2011 for review). Additionally, in nestlings increased CORT levels that result from nutritional deficiency can cause increased begging that may, in turn result in increased feeding by the parents (Kitaysky et al., 2003; Louiseau et al., 2008). In contrast, it might be predicted that if a nestling is well-supplied

with food through supplemental feeding, that an individual would beg less and have lower CORT levels than nestlings that are not receiving supplemental food.

We considered the relationship among food availability, plasma CORT levels, and begging behavior in the Florida scrub-jay (*Aphelocoma coerulescens*, FSJ). In treatment nests, we indirectly supplemented all nestlings within a brood by providing FSJ parents with ad libitum supplemental food suitable to feed to nestlings (mealworms) during the nestling period. Control nests received no manipulation besides normal nest monitoring. Due to the poor success of control nests, we have also included nests that were used in a separate study (Chapter 1) in which a single nestling per nest was treated with CORT. We predicted that nestlings provided with supplemental food would 1) beg fewer times and for a shorter duration and 2) have lower baseline and stress response CORT levels on day 11 post-hatch compared to individuals that did not receive supplemental food.

## **Methods**

### *Study System*

This study was performed during the breeding season of 2012 on a well-studied, free-living population of Florida scrub-jays at Archbold Biological Station in south-central Florida (27°10'N, 81°21'W). FSJs are non-migratory, socially and genetically monogamous, and live in family groups that average three jays (Woolfenden and Fitzpatrick, 1984; Quinn et al., 1999; Townsend et al., 2011). Each bird in the population is uniquely color-banded and monitored from hatching until death. FSJs begin nesting in early March with a mean clutch size of three eggs and incubation and nestling periods that both average 18 days. FSJ nestlings are altricial and rely on parents and non-breeding “helpers” for protection and nourishment while in the nest and until nutritional

independence at approximately 70 days post-hatch (Woolfenden and Fitzpatrick, 1984). Approximately half of the breeding pairs in our population have from 1-6 helpers with a mean group size of 3.

All FSJ nests in the population were located during the nest building stage and monitored until the nest was abandoned, depredated, or successfully fledged young. Nests were randomly assigned to a treatment prior to the season. Frequent nest checks on the predicted day of hatching allowed us to record hatch order and uniquely mark a toenail of each nestling and determine their mass at hatching. Toenails were repainted on days 3, 5, and 8 post-hatch, thereby allowing individuals to be monitored throughout the nesting period. On day 5 post-hatch, nestlings were reweighed and a tarsus measurement was taken. On day 11, the nestlings were banded with a single plastic color band and an U.S. Geologic Survey aluminum band and we measured individuals' tarsus, wing length, and body mass. Body measurements collected on hatch day, day 5, and day 11 were used to calculate growth rate of all individual nestlings. Growth rate was calculated as the daily increase in mass from hatch to day 11.

#### *Blood Sampling*

Blood samples from all nestlings were collected on day 11 post-hatch. To ensure that a baseline CORT sample was obtained from each nestling, all nestlings were removed from the nest simultaneously (time 0) and multiple researchers were present to take blood samples. For all individual nestlings, a small blood sample (~100  $\mu$ l) was taken from the brachial vein within three minutes of removal from the nest (Romero and Reed, 2005). Subsequently, nestlings were held in the hand until 10 minutes post nest removal when we took a second blood sample (~100  $\mu$ l) to determine a stress-induced

CORT level. Blood samples were placed on ice until return to the lab (within an hour of blood sampling) where they were centrifuged for 5-7 minutes at 12000 rpm. The plasma was drawn off and frozen until analysis at the University of Memphis.

Samples were assayed using the Cayman 500655 Corticosterone Enzyme-Linked Immunosorbent Assay (ELISA; Cayman Chemicals, Ann Arbor, MI) following the recommended protocol. We used three plates to run all samples on the same day. In all instances, baseline and stress induced samples from all individuals within a nest were run on the same plate. Baseline samples were diluted 1:20 in EIA buffer and stress-induced samples were diluted 1:100 in EIA buffer prior to assay. These dilutions were used to assure binding values fell within the linear portion of the sigmoidal standard curve (i.e., between 15 and 65% bound). CORT standards of known concentration were included on each plate (intra-assay CVs  $\leq 3\%$ ; inter-assay CV  $\leq 5\%$ ).

#### *Supplemental Feeding*

We used Radio Frequency Identification (RFID) enabled *SmartFeeders* (see Bonter and Bridge, 2011) to allow select individuals access to supplemental food. Each individual adult FSJ has a Passive Integrated Transponder (PIT) tag affixed to a leg band. The RFID technology recognizes an individual's identity by reading the PIT tag when the individual lands on the feeder. Adults at a subset of territories ( $n = 6$ ) were supplemented with peanuts during the pre-breeding season. We used this technology to indirectly supplement all nestlings within selected broods by providing ad libitum mealworms during the nestling period ("feeder nests"). One to three days prior to the predicted hatching day, the feeders were modified to deliver live mealworms. Mealworms were provided until nestlings fledged or the nest failed. Previous observations found that ~50%



of the mealworms taken from the feeder were fed to nestlings (Small and Schoech, unpublished data).

#### *Corticosterone Treatment*

In addition to the few unmanipulated control nests that successfully raised young to day 11, we included data collected from nests used in a separate experiment investigating the effects of CORT on begging behavior and growth (see Chapter 1). A single nestling in a selected subset of territories received exogenous CORT for a period of 3.5 days from day 8 post-hatch to the morning of day 11. Refer to the methods section of chapter one for specific details on the CORT administration. No effect of CORT on begging behavior was found in that experiment, so we included all nestlings within those nests as controls for the food supplemented groups in this study.

#### *Nest Monitoring*

We used *TowerCams*, a high definition video camera (JVC HD Everio, GZ-HM440) mounted atop a 1.9 meter pole that can extend up to 3.2 meters, to monitor nest behavior. Most FSJ nests are 1-2 meters off the ground (Woolfenden and Fitzpatrick 1984) and the vantage point of the cameras allowed us to monitor individual nestling behavior as well as adult behavior from a distance that did not disturb the adult jays. A preliminary study in 2011 found that adult behaviors were not altered by the presence of a camera (Elderbrock, unpublished data). Recording began between 7:30 and 11:00 on days 5, 8, 10 or 11, and 13 post-hatch. Filming for the nests included in the CORT-treatment study was on day 10 following the 5<sup>th</sup> treatment. All other nests were filmed on day 11 prior to blood sampling. The data for these two days were analyzed together and considered as “day 11”. The camera would be set up and turned on and the individual

nestlings would be identified in the nest by their toe mark and then immediately after on camera by their position in the nest. Because I knew the position of each individual nestling at the beginning of recording, I could assess begging behavior and parental feeding for each individual for the duration of the video. The nests were filmed for 90-120 minutes. The variation in recording duration was due to limited camera availability when videoing at multiple nests in a single morning and because some sessions were shortened by rain. The lack of standardization in recording duration has been compensated for in the analyses by expressing variables as rates per hour.

#### *Video Analysis*

Videos were used to monitor both nestling and adult behaviors. From each recording we measured the number of begs and duration of begging bouts, as well as the number of times each individual nestling was fed. A “beg” was counted every time a nestling raised its head and opened its beak. The “beg duration” was the average time in seconds a nestling kept its beak open. Each beg received a minimum duration of one second. A “feed” was counted when an adult put food into an individual’s open beak and the individual swallowed the food. Each variable was converted to a rate per hour per individual for statistical analyses.

#### *Statistical Analysis*

We compared three nest groups for the analyses: supplementally fed (“feeder nestlings”), non-manipulated controls (“control nestlings”) and CORT-treated (“CORT nestlings”). To meet assumptions of normality, the number of begs, feeds and beg duration were square root transformed. We used a two-way repeated measures analysis of variance (ANOVA) for each of the three variables separately and, when appropriate, used

the Holm-Sidak post hoc method to compare across the four observation days and between the three treatment groups. Although there was no interaction between observation day and treatment, because CORT-treatment in the CORT nests did not start until three days after the first day of behavioral monitoring (chapter 1), we used the Holm-Sidak post hoc test to compare between groups on each day. Baseline CORT levels were log transformed to meet assumptions of normality. We used two-way repeated measures ANOVAs to compare baseline and handling stress (stress-induced) CORT levels between the three groups on day 11 post-hatch. SigmaPlot 12.0 and PASW 18 were used for statistical analyses.

## **Results**

### *Begging Rate*

Begging rates differed among days ( $F_{(3,148)} = 10.41, P < 0.0001$ ) and rates were greater on day 13 than day 5 ( $P < 0.0001$ ), day 8 ( $P < 0.001$ ) and day 11 ( $P = 0.018$ ). There were no other differences between days (all  $P > 0.05$ ). Begging rate also differed among the three nest groups (Fig. 1,  $F_{(2,148)} = 9.64, P = 0.0001$ ) with feeder nestlings begging significantly less than the CORT nestlings ( $P < 0.001$ ) but not the control nestlings ( $P = 0.44$ ). There was no difference between feeder and control nestlings ( $P = 0.48$ ). Although there was no day\*treatment interaction ( $F_{(6,148)} = 1.474, P = 0.20$ ), the Holm-Sidak post hoc tests were used to determine if begging rates changed in CORT nests in response to the initiation of CORT-treatment on day 8. No difference was found in the number of begs on day 5 (pre-treatment) or day 8 (first day of treatment;  $P > 0.05$ ). However, feeder nestlings begged less than the CORT nestlings on day 11 ( $P = 0.013$ ).

and day 13 ( $P = 0.001$ ). Control nestlings did not differ from feeder or CORT nestlings on either day ( $P > 0.05$ ).

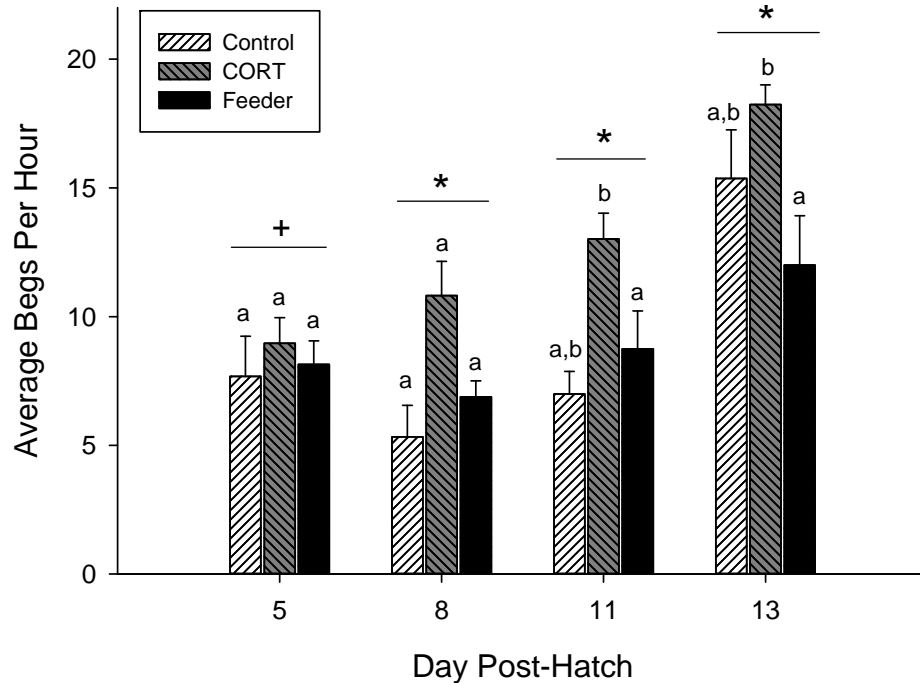


Figure 1. Mean number of begs per hour in treatment nests across the four days of behavioral observations. Different letters indicate significant differences among treatments within a particular day. Different symbols (\*, +) indicate difference among days (all treatments combined). Error bars indicate standard error.

### *Beg Duration*

Begging duration differed among days (Fig. 2,  $F_{(3,148)} = 4.88$ ,  $P = 0.003$ ) with nestlings begging longer on day 11 than on day 5 ( $P = 0.003$ ). However, there was no treatment effect (Fig. 2,  $F_{(2,148)} = 0.97$ ,  $P = 0.384$ ) and no day\*treatment interaction ( $F_{(6,148)} = 1.67$ ,  $P = 0.14$ ).

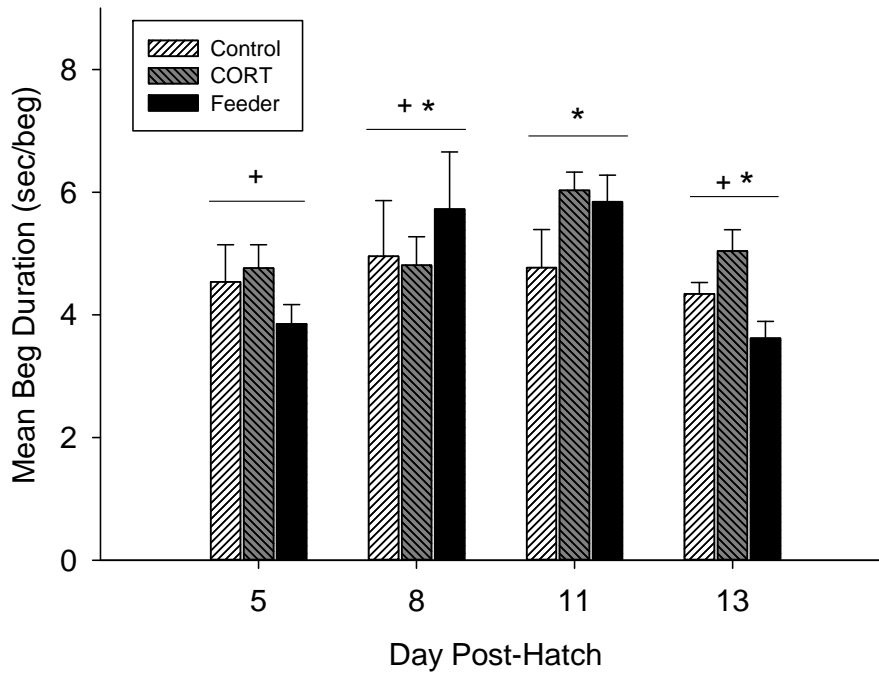


Figure 2. Average begging duration (seconds/beg) in treatment nests across the four days of behavioral observations. Different symbols (\*, +) indicate difference among days (all treatments combined). Error bars indicate standard error.

### *Parental Feeds*

There was no difference in the number of parental feeds to individual nestlings among nest types ( $F_{(2,148)} = 0.051$ ,  $P = 0.95$ ), or observation days ( $F_{(3,148)} = 0.91$ ,  $P = 0.44$ ). There was no day\*treatment interaction ( $F_{(6,148)} = 0.98$ ,  $P = 0.44$ ).

### *Corticosterone Levels*

Overall, nestlings exhibited a significant increase in CORT levels with handling time (Fig. 3,  $F_{(1,71)} = 54.80$ ,  $P < 0.0001$ ). However, there was no difference among treatments ( $F_{(1,71)} = 1.86$ ,  $P = 0.17$ ) nor was there a treatment\*time interaction ( $F_{(1,71)} = 0.014$ ,  $P = 0.99$ ). Nestlings in all three groups exhibited highly significant increases in CORT levels with handling time (feeder [ $P < 0.001$ ], control [ $P = 0.005$ ], and CORT [ $P <$

0.001]). However, no differences were found between the three nest types at either time point (all  $P > 0.05$ ).

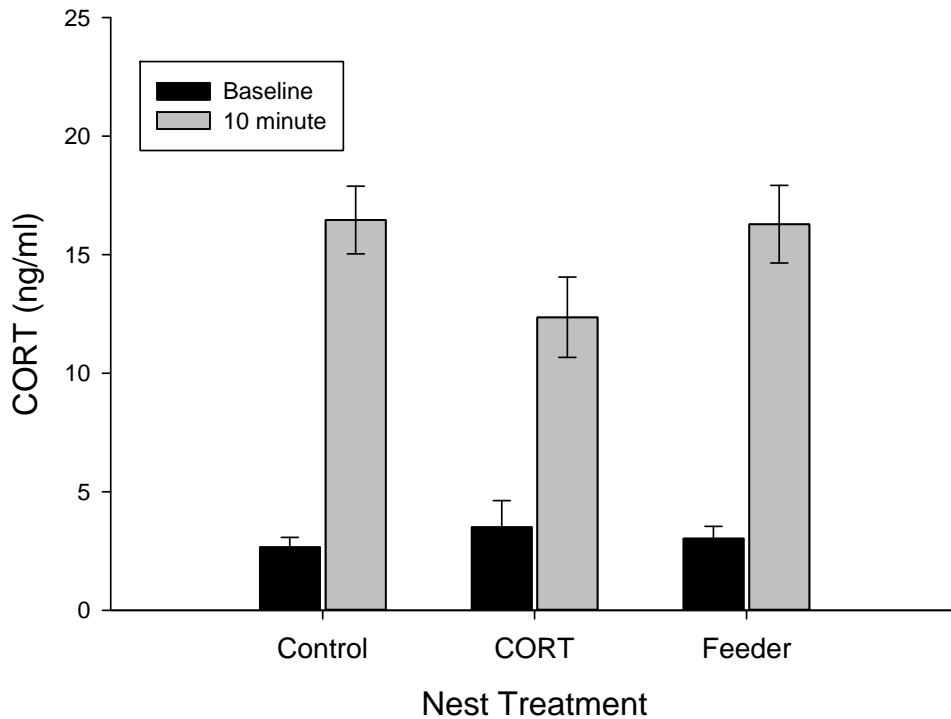


Figure 3. Mean baseline and stress-induced CORT level means by treatment. Bars indicate standard error ( $n = 3, 21, \text{ and } 13$  nestlings, respectively).

## Discussion

The primary aim of this study was to investigate the potential impact of supplemental feeding on begging behaviors and plasma CORT levels in FSJ nestlings. We found minimal support for supplemental feeding having an influence on begging behavior, adult feeding behavior, or plasma CORT levels. A significant difference was found in begging rate between feeder nestlings and CORT nestlings, but we believe this is due to the CORT treatment, rather than supplemental feeding.

The breeding season of 2012 was poor for all FSJs in the population (see Schoech 2009 for a discussion of ‘good’ vs. ‘bad’ years in this system). Overall, only 39 out of 49 pairs (79.6%) attempted to nest and only 57 nestlings survived to day 11. In comparison, 100% of established pairs in 2010 (45 pairs) and 2011 (53 pairs) nested and 113 and 129 nestlings survived to day 11, respectively. This uncharacteristically poor year was likely due to drought conditions during the preceding winter and pre-breeding season. Adult FSJs feed their offspring a diet of insects (Woolfenden and Fitzpatrick, 1984) and under drought conditions, the insect populations are almost certainly lower. Similar drought years, in which a relatively large number of pairs have forgone breeding and overall production of offspring was poor have been recorded for this population (Schoech, 1996, 2009). Seemingly, such conditions would provide an opportunity to investigate the effects of supplemental food on begging behavior and the CORT response in FSJ nestlings. Because of the poor year, we anticipated that supplemental food, both prior to and when nestlings were present, would simulate conditions roughly equivalent to a ‘good’ year for both adults and nestlings. In contrast, we expected the non-supplemented groups (CORT and control nestlings) would have limited access to resources and this would be reflected in increased begging relative to the nestlings in food supplemented nests. We hypothesize that under extremely poor environmental conditions, the beneficial effects of supplemental food are insufficient to overcome the poor conditions.

Supplemental feeding did not alter the behavior of the nestlings or parents as expected. Beg rate, beg duration, and the number of feeds were no different between feeder nestlings and non-manipulated controls. However, because of the poor season there was little success in the control nests (nests that did not receive supplementation or

other manipulation). Due to the low sample size for control nests, drawing conclusions from the lack of a difference between feeder and control nests is problematic until further data are collected. The low number of control nests is attributable to two primary factors. First, assignment of nests to feeder and CORT-treatment was given priority early in the season. Second, the low nesting rate deprived us of another 10 potential control nests as 20% of the established pairs did not breed.

Somewhat surprisingly, overall the nestlings in the CORT-treated nests begged at a greater rate than feeder nestlings (Fig. 1). The data suggest that these differences are an outcome of the CORT nestlings begging more on average, rather than feeder nestlings begging less as we had originally predicted. This is revealed by the increased differences in mean begging rate between feeder and CORT nestlings after the CORT-treatment was started on day 8 post-hatch (see Fig. 1). Although begging rate differed, the average duration of each beg did not differ between groups (Fig. 2). Thus, CORT nestlings begged more often, but each individual beg was not significantly longer than the feeder nestlings.

Supplemental feeding typically decreases CORT levels in free-living adult birds (Schoech et al., 2007; Kitaysky et al., 2010) and CORT levels are higher in nutritionally restricted nestlings (Kitaysky et al., 2006; Pravosudov and Kitaysky, 2006). Thus, we predicted that both baseline and stress-induced CORT levels would be lower in the feeder nestlings than the non-supplemented nestlings. However, supplemental feeding did not impact CORT levels at either time-point and there were no differences in CORT levels among the CORT, feeder, and control nestlings. This suggests that food supplementation



did not improve nestling quality, at least in a manner that allowed us to detect differences in the considered measures of nestling behavior and physiology.

It is possible that natural food availability was not as poor as anticipated this season, thus diminishing the advantage of supplemental food. In the past, FSJ groups provided with supplemental food during the pre-breeding season have usually bred earlier than groups without supplementation (Schoech, 1996; Schoech et al., 2004; but see Schoech et al., 2008). However, during the breeding season of 2012, supplemental food did not result in earlier breeding (Small and Schoech, unpublished data). All groups initiated breeding much later than average (of 40 years of study, 2012 was the latest year on record, R. Bowman, personal comm.). Another explanation for the failure of supplementation to affect the timing of breeding and nestling and adult behavior in this study may be that the positive stimulus of food supplementation could not overcome the poor environmental conditions. Alternatively, our inability to detect a difference in timing of reproduction between feeder and non-supplemented control groups, despite relatively large differences in mean begging rates, may be simply due to the small sample size of control groups.

In addition to nestling behavior, we also did not find a difference in adult behavior as feeding rates of the adults did not differ between the three nest types. Regardless of the amount of food available to the parents, they did not modify their feeding rate. This suggests that parental behavior is somewhat independent of resource availability but rather is controlled by offspring behavior. Given an unlimited supply of nestling-suitable food, if the supplemented parents were driven by a 'desire' to produce the fattest (i.e., most fit) young, they might have been expected to feed young constantly.

Instead, they appear to bring food only in response to begging. However, even though the nestlings begged more in the CORT-treated nests, their parents did not feed at an increased rate. Although speculative, it may be that even with increased begging, parents were incapable of responding to the nestlings given the poor environmental conditions and accompanying limited food availability. Future study in which CORT-treatment is combined with food supplementation may address this speculation.

### **Conclusion**

In conclusion, food supplementation had no detectable or direct effect on nestling begging behavior, adult feeding behavior, or nestling CORT levels in FSJs. Additionally, even though it was not one of the main aims of this study, our results reveal an intriguing indirect effect of CORT-treatment upon begging behavior in FSJ nestlings. While we found no behavioral differences between CORT-treated and control nestlings within a nest (see Chapter 1), it is possible that sibling competition is driving behavioral modifications for all nestlings in the CORT nests (Godfray and Johnstone, 2000). For example, CORT-treatment leads to increased begging by treated individuals and this, in turn, induces non-treated nest-mates to increase begging rates to compete for parental attention. Data collection in future field seasons will help decipher the effects of supplemental feeding and CORT-treatment on nestling and parental behavior, as well as nestling plasma CORT levels.

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## CHAPTER 4: CONCLUSION

In conclusion, I found little support that supplemental food had an impact on nestling begging or plasma CORT levels. In addition, I found no difference in behavior or growth of the nestlings that were treated with CORT, although the treated nestlings had significantly higher CORT levels as a result of being fed the CORT-injected waxworm. Unexpectedly, in the second study, I found evidence that the CORT-treatment had an effect on begging. When I considered the average begging rate of all nestlings within the CORT-treatment nests, there was a significant difference between the CORT-treated nestlings and the feeder nestlings. This was unexpected because the control-fed individuals did not have increased CORT levels (see Chapter 1). We hypothesize that this change in behavior of all nestlings within the CORT-treated nests was a result of interaction and competition between nestlings.

Sibling competition is within-nest competition for limited resources delivered by parents (Godfray and Johnstone, 2000). Although speculative, the increased overall begging in CORT nests may be attributable to increased begging by the CORT-treated nestlings that effectively induced increased competition (begging) by the non-treated siblings. Testosterone is known to be involved in aggression and to be correlated with increased begging of nestlings of some species (Goodship and Buchanan, 2007; Smiseth et al., 2011). Further study that incorporates consideration of testosterone levels would help clarify the factors driving begging behavior in this species.

This is the first study to investigate begging behavior within individual Florida scrub-jays. Our results suggest that CORT may play a role in the regulation of begging and additional research will focus on this behavior at more specific time-points following

treatment (i.e., address the behavior immediately post treatment given the relatively short duration of the treatment, see Schoech et al., 2007). In addition, if a single CORT-treated nestling is driving competition, further analysis of which nestling initiates begging upon a parental visit to the nest might confirm this speculation. Although there is evidence that nutritional deficiency causes an increase in CORT levels, we found that supplemental food did not lower plasma CORT levels or alter begging behavior. Future research that combines food supplementation and CORT manipulation may be revealing.



## IACUC PROTOCOL ACTION FORM

<b>To:</b>	Stephan Schoech
<b>From</b>	Institutional Animal Care and Use Committee
<b>Subject</b>	Animal Research Protocol
<b>Date</b>	5-15-2009

**The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. 0667 (COLLABORATIVE RESEARCH: ...)**

Your proposal is approved for the following period:

From:  To:

Your protocol is not approved for the following reasons (see attached memo).

Your protocol is renewed without changes for the following period:

From: \_\_\_\_\_ To: \_\_\_\_\_

Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Revision Memorandum dated \_\_\_\_\_ for the following period:

From: \_\_\_\_\_ To: \_\_\_\_\_

Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Revision Memorandum dated \_\_\_\_\_

---

Prof. Guy Mittleman, Chair of the IACUC

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Dr. Karyl Buddington, University Veterinarian  
And Director of the Animal Care Facilities





## IACUC PROTOCOL ACTION FORM

<b>To:</b>	Steve Schoech
<b>From</b>	Institutional Animal Care and Use Committee
<b>Subject</b>	Animal Research Protocol
<b>Date</b>	2-24-2011

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. Transdermal CORT (0697)

Your proposal is approved for the following period:

From:  To:

**Protocol requires approval notice from Florida Biological Station.**

Your protocol is not approved for the following reasons (see attached memo).

Your protocol is renewed without changes for the following period:

From: \_\_\_\_\_ To: \_\_\_\_\_

Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Revision Memorandum dated \_\_\_\_\_ for the following period:

From: \_\_\_\_\_ To: \_\_\_\_\_

Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Revision Memorandum dated \_\_\_\_\_

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Prof. Guy Mittleman, Chair of the IACUC

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Dr. Karyl Buddington, University Veterinarian  
And Director of the Animal Care Facilities

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## NOTICE OF ARCHBOLD IACUC REVIEW

Date received: 10/12/2011

Assigned Protocol #: ABS-AUP-005-R

The Committee has reviewed this protocol and our decision is to:

**Approve the AUP as is.** The project may begin. Investigators must adhere to the conditions outlined below.

**Conditionally approve the AUP pending revision.** See attached IACUC comments and revise your protocol application accordingly (full committee review will not be required to act on the revised application). The project may not begin until approval is obtained.

**Delay action due to insufficient information.** The investigator(s) must completely fill out all relevant sections of the Archbold Animal Use Protocol Application and resubmit the application to the Chair of the Archbold IACUC (full committee review may be required to act on the application).

**Denied.** The proposed project may not be conducted at Archbold.

Date of Action: 11/9/2011

Approval expires on: 12/31/2012 unless PI or Instructor requests renewal (see below).

Signature of Committee, Archbold IACUC

Date

Signature of Chairperson, Archbold IACUC

Date

### Conditions:

**\*\*Because of the novelty of this research, we are granting a 1-year approval and request that the PI provides us (at the end of the first year) with empirical evidence demonstrating that the CORF-supplementation to nestlings has no adverse effects. This information should be submitted with the Annual Review Form and will be used as the basis for issuing continued IACUC approval for future years. Also note the following:**

- Investigators must conform to all federal and state laws, guidelines, and all institutional policies and procedures concerning the care and use of animals.
  - Investigators must notify the Archbold IACUC in writing (email is acceptable) of any changes in the proposed project, personnel, number of animals, or the type of animals prior to proceeding with any further animal use.
  - The Investigator must provide to the IACUC, by the annual expiration date indicated above, an annual report itemizing the animals used in each approved protocol.
  - If the project described in this application is used as a basis for a proposal for funding, the Investigator must ensure that the description of animal use in such a funding proposal is the same as that contained in this application. If the funding proposal deviates significantly from the work described herein, then the Investigator must submit a revised application to the IACUC which describes the differences.
  - The Investigator must notify the IACUC, in writing, if animals used for this protocol are transferred to another investigator for use in a different protocol.
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