EFFECTS OF SUPPLEMENTAL FEEDING ON STRESS

PHYSIOLOGY AND NESTING SUCCESS OF

EASTERN BLUEBIRDS, *SIALIA SIALIS*

By

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Abstract: A common anthropogenic influence on wildlife is the use of supplemental bird feeders. Dependent on abundance and natural food availability, this supplemental food source could influence individual survival and productivity. In this study, supplemental food availability was experimentally manipulated in a wild population of Eastern Bluebirds, *Sialia sialis.* This was done to examine the influence of the common pastime of bird feeding on physiology and reproductive success of bluebirds, especially when supplemental feeding is inconsistent. Adult and nestling bluebirds were assigned to one of three groups. In the first group, birds received mealworms (*Tenebrionidae* larvae) throughout the breeding attempt. In the second group, birds received mealworms from nest completion until nestlings hatched. Birds in the third group received no supplementation but were disturbed at the same frequency as birds in the other two groups. Nestling growth and reproductive success were calculated to examine differences resulting from my experimental manipulation. I also collected blood samples from adults and nestlings to quantify differences in bacterial killing ability, circulating corticosterone levels, and heterophil to lymphocyte ratios between the experimental groups. Finally to determine if differences in habitat quality contributed to the effect of food supplementation on bluebird physiology and nest success, data on invertebrate abundance were collected on a subset of territories. I found bacterial killing ability, baseline corticosterone and heterophil to lymphocyte ratios of adults and nestlings were not significantly different across the experimental groups. Nestling mass, tarsus, and wing chord length were unaffected by experimental treatment. Invertebrate abundance and richness were similar between years as well as across nest box trails. Invertebrate abundance and richness were not correlated with any of the nesting success metrics and did not statistically influence nesting success. Experimental manipulation of supplemental feeding did not appear to influence physiology or nesting success of Eastern Bluebirds. With regard to immune function, it is possible female condition prior to egg laying has a greater influence on nestlings than changes in food availability post-laying and later. Supplemental food availability may only have significant effects on physiology metrics and nest success in years with low environmental food availability.

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CHAPTER ONE

GENERAL INTRODUCTION

INTRODUCTION AND OBJECTIVES

Anthropogenic effects on wildlife are important to consider as these effects can have a lasting influence on populations. A particularly common anthropogenic effect is the addition of supplemental food to an animal's environment. Supplemental food provisioning for birds is particularly common, especially in urbanized areas (Robb et al., 2008). Waste around cities can assist generalist and invasive species such as House Sparrows (*Passer domesticus*) by providing additional food sources including grains and seeds. Research has been conducted previously on the effects of consolidated waste in city landfills on bird populations (Kihlman and Larsson, 1974). The study evaluated wintering populations of Herring Gulls (*Larus argentatus*) and found that the landfill in the research area increased the local population size. Several other studies have also investigated effects of landfills (Boarman et al., 2006; Duhem et al., 2008). While population sizes of species tolerant to urban environments generally increase in density, it is typically at the expense of biodiversity (Shochat et al., 2010).

Bird feeders, or supplemental feeders, are common in urban environments throughout North America with $3.4 billion dollars spent on supplemental bird feed, and 47% of households participating in the pastime in 2006 (Orros and Fellowes, 2012; USFWS, 2006). In my thesis, I used an experimental approach to test the effects of supplemental feeding on a wild population of Eastern Bluebirds (*Sialia sialis)* a common target for bird feeding, by addressing three objectives:

OBJECTIVE ONE: *Determine how access to supplemental food impacts baseline corticosterone (CORT) concentrations, heterophil to lymphocyte ratios (H:L) ratios, and bactericidal capabilities of Eastern Bluebirds.*

OBJECTIVE TWO: *Determine how access to supplemental food influences nesting success of Eastern Bluebirds.*

OBJECTIVE THREE: *Determine how natural food abundance in the environment interacts with supplemental feeding to influence metrics of nesting success.*

METHODS OVERVIEW

I manipulated food availability for Eastern Bluebirds by creating three trial groups. The first group was given mealworms as a supplemental food source from the nest construction phase until the nestlings fledged (FULL). The second group was given supplemental food from nest completion until egg hatching (PART). These groups were then compared to the control (CONT), which did not receive supplementation but, in which nest boxes were opened the same number of times as the FULL and the PART groups.

To accomplish my objectives, I used two metrics of activation of the stress response. First, I evaluated concentrations of the steroid hormone corticosterone (CORT). CORT is the main glucocorticoid in birds, and is released in response to stressors, including reduced food availability (Wingfield, 1995). Secondly, I quantified the leukocyte profile, particularly the ratio of heterophils to lymphocytes (H:L ratio) in circulation. In response to stress exposure, the number of heterophils in circulation generally increases and the number of lymphocytes decreases (Davis et al., 2008). This change in the leukocyte profile occurs more slowly than the elevation of circulating CORT and may also persist over a longer period of time (Davis et al., 2008; Goessling et al., 2015). Lack of food, or removal of a food source at a critical time point in the breeding cycle, may be a stressor sufficient to affect these metrics in birds. I also evaluated plasma bactericidal capabilities through baterial killing assays (BKA). Bactericidal capability is a useful metric for evaluating an individual's susceptibility to infection and potential ability to mount an immune response (O'Neal and Ketterson, 2011).

To evaluate nesting success, I recorded clutch size, hatching success, brood size and fledging success in each of the experimental groups. I also conducted invertebrate surveys to approximate natural food availability in the environment. I then compared the nest success metrics with my data on natural food abundance, and invertebrate order richness to determine if the amount of food available in the environment impacted the response of individuals to my supplement groups. Overall, this study evaluated the effects of an anthopogenic food source on a species commonly targeted for supplemental feeding and thus provides insight into human impact on a species.

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CHAPTER TWO

EFFECTS OF SUPPLEMENTAL FEEDING ON EASTERN BLUEBIRD

PHYSIOLOGY

INTRODUCTION

Human populations coexist with wildlife populations; therefore, it is important to

understand both negative and positive interactions we have with wildlife populations. A

perceived beneficial anthropogenic effect on wildlife populations is supplemental

feeding, which is an additional anthropogenic food source in an environment, when it is

not naturally available (Robb et al., 2008a; Sguassero et al., 2012). Previous studies have

shown that supplemental feeding can increase bird population numbers and advance

laying date (Ewald and Rohwer, 1982; Galbraith et al., 2015). However, the

physiological basis for the effects of supplemental feeding on population numbers and

mortality have been studied less extensively (Arcese and Smith, 1988; Brittingham and

Temple, 1988). Additionally, it is possible that earlier laying dates and larger population

sizes are not beneficial over the long-term. This may be true if some birds that survive as

a result of supplemental feeding are ultimately not able to successfully compete for

nesting sites (Jones et al., 2014). Moreover, earlier egg laying may lead to a mis-match

between the peak of natural food availability and the peak of offspring food demands (Robb et al., 2008b; Schoech and Bowman, 2001). Additionally, among birds, the use of supplemental feeders can have ecologically and physiologically detrimental impacts, including increased risk for predation exposure and disease transmission due to congregation at feeders (Hanmer et al., 2017; Robb et al., 2008b). Metrics that allow us to uncover any detrimental effects on wildlife caused by supplemental feeding would be extremely valuable in this context.

To evaluate physiological condition, some metrics are easily quantifiable, indirect measures of potential survival probability (Bonier et al., 2009; Davis et al., 2008; Marra and Holberton, 1998). These indirect measures include circulating levels of the steroid hormone corticosterone (CORT) which, when chronically elevated may be associated with reduced survival between years (Goutte et al., 2010) and lower immune function (Angelier et al., 2010). However, the relationship between CORT and individual condition or allostatic load is complicated, particularly in the context of field studies, as many factors can trigger the release of CORT (Bonier et al., 2009; Goutte et al., 2010). Another indicator of survival probability is the change which occurs in the leukocyte profiles of individuals, particularly the ratio of heterophils to lymphocytes (H:L ratio), in response to stressors (Lobato et al., 2009). Nestlings with lower total leukocyte counts have higher recruitment rates, as was seen in pied flycatchers, *Ficedula hypoleuca* (Lobato et al., 2009). Also heightened H:L ratios can indicate exposure to ectoparasites (Müeller et al., 2011) or recent injury (Vleck et al., 2000), and are modulated by stress hormone levels (Davis et al., 2008). A final indicator of survival probability is a functional measure of immune function known as bacterial killing ability (Hornef et al.,

2002). Bactericidal capabilities are good indicators of an individual's susceptibility to bacterial infection (Millet et al., 2007; O'Neal and Ketterson, 2011; Tieleman et al., 2005).

CORT is one hormone involved in moderating the stress response in vertebrates, and sends signals to the body to respond to stress stimuli (Wingfield, 1995). Stress coping methods include redistribution of white blood cells to attack pathogens and heal wounds (Dhabhar et al., 2009). CORT secretion has been shown to increase in times of food strain or experimental fasting (Kitaysky et al., 2007; Lynn et al., 2003; Lynn et al., 2010). Food strain can be induced by low food availability in the environment or the removal of a food source at a critical time during reproduction. It is possible, therefore, that birds with supplemental food available would have lower baseline levels of CORT due to reduced food strain. A study in another passerine bird, the Eurasian Treecreeper (*Certhia familiaris*), found a negative correlation between invertebrate density, as a measure of food availability, and CORT concentrations (Suorsa et al., 2003). However, CORT has a short half life, approximatey 22 minutes in chickens (Birrenkott and Wiggins, 1984), which causes the hormone to dissipate fairly quickly after an acute stressor is removed. For stressors of longer duration, such as chronic food stress, baseline CORT levels may not be a reliable indicator of stress exposure (Kitaysky et al., 2007; Smith et al., 2006). Also, simulations of natural environmental changes including food manipulation can have different outcomes than other types of chronic stressors, indicating study design may in itself influence CORT results (Dickens and Romero, 2013). Therefore, another metric of an elevated stress response, such as changes in the leukocyte profile, may be

more responsive to (Mashaly et al., 2004; Maxwell et al., 1993), or better indicate, prolonged stress exposure (McFarlane and Curtis, 1989; Vleck et al., 2000).

In birds, heterophils attack and kill pathogens through phagocytosis (Genovese et al., 2013). Lower heterophil numbers indicate that cells have not been recently triggered by an inflammatory response or foreign bacteria (Davis et al., 2008). Lymphocytes also function in systemic pathogen defense and include T cells, B cells, and Natural Killer cells (Berrington, 2005). In response to stress exposure, the number of heterophils in circulation increases and the number of lymphocytes decreases, increasing the H:L ratio overall (Davis et al., 2008). This change in the leukocyte profile occurs more slowly than the elevation of circulating CORT levels and may also persist over a longer period of time (Davis et al., 2008). Thus, this measure of innate immune function may provide important insight into the effects of food stress over the long-term.

Supplemental feeding may also contribute directly or indirectly to disease susceptibility. Individuals at bird feeders may be at risk of increased risk of exposure to diseases, such as *Salmonella* and avian pox, due to congregation around the feeder site (Brittingham and Temple, 1986; Fischer et al., 1997; Robb et al., 2008b; USGS, 2016). It is possible that supplemental feeding can prolong the lives of diseased individuals, which otherwise may not have been able to forage (Fischer et al., 1997). It is important to understand if individuals at supplemental feeders have enhanced immune function either through repeated exposure to disease or increased energy available to allocate toward immune function. Bactericidal capability as measured with bacteria killing assays (BKA) is a good direct measure of constitutive immune function against specific bacterial strains, and is easily interpretable because a greater level of killing in the assay indicates

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greater resistance against bacterial infection (Matson et al., 2006; Millet et al., 2007; O'Neal and Ketterson, 2011).

I studied the potential effects of supplemental feeding on stress physiology and immune function of Eastern Bluebirds, *Sialia sialis*. Eastern Bluebirds are an insectivorous and invertebrate consuming passerine species that are commonly provided with mealworms (*Tenebrionidae* beetle larvae) as a supplemental food source. The species' range extends from the central United States to the Atlantic Coast (Sibley et al., 2014), and birds prefer to nest in edge habitats, including edges created by human development, which brings them into close contact with humans (Jones et al., 2014). Eastern Bluebirds are secondary cavity nesters, utilizing both natural and artificial cavities.

I manipulated food availability for Eastern Bluebird adults and nestlings in one of three ways. A fully supplemented nesting attempt received mealworms from nest completion until fledging (FULL). The partially supplemented group received mealworms only until hatching (PART), and these two groups were compared to a control (CONT), which received no mealworms, but was disturbed at the same frequency. All boxes were opened the same number of times, regardless of treatment group. Further, I provided supplementation inside of the boxes to be able to specifically manipulate food availability for particular individuals, which differs from most externally mounted feeders used in previous studies (i.e. Brittingham and Temple, 1986). I hypothesized, that CORT and H:L ratios would be higher in birds more subject to food strain (PART>CONT>FULL). This would be due to the fact that of my experimental groups, the PART group may be the most subject to food strain as this manipulation

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removes a food source at a time when food requirements rapidly increase (hatching stage). Finally, I hypothesized that the bacterial killing ability of nestlings would be highest for young in nest boxes with supplemental food available throughout the nesting attempt (FULL>PART/CONT), as supplemental feeding of adult females prior to egg-laying has been shown to improve other metrics of nestling immune function (Moreno et al., 2008). Due to the short period of time between food manipulation and blood sample collection in my study, I did not anticipate differences in H:L and BKA between the FULL and PART groups in the adults. If there were a difference to be seen in H:L and BKA, I would predict that the FULL/PART groups would have higher BKA due to increased energy availability to devote towards immune function, and lower H:L ratios due to the reduction of any potential food strain the adults faced naturally. I also predicted that BKA would be higher in adults than in nestlings because younger birds cannot yet efficiently produce antibodies endogenously (Grindstaff et al. 2003), and are largely limited therefore, to innate immune responsiveness (Lawrence et al., 1981; O'Neal and Ketterson, 2011).

METHODS

Field methods: Nest boxes (2015: n = 187, 2016: n = 176) located along walking trails and roads in Stillwater, Oklahoma (36°7′18″N 97°4′7″W) were used to manipulate the food available to breeding Eastern Bluebirds. Nest boxes were checked 2-3 times weekly throughout the breeding season from March-August in 2015 and 2016. Individual boxes were monitored daily when complete nests were detected to determine first egg dates. Boxes were again monitored daily when clutches were within two days of their expected hatch date (clutch completion date + 13 days). Then finally, nest boxes were also

monitored daily when nestlings were 14 days post-hatch or older to determine if fledging occurred. This level of monitoring follows methods used previously in this population (e.g., Grindstaff et al., 2012). Small feeding cups (2 oz., plastic) were mounted in all nest boxes prior to the breeding season. In these cups, supplemental food (mealworms) was provided by adding fifteen mealworms (1.909 ± 0.177g) per individual bluebird in the nest box, including both adults and nestlings, three times per week (Smith, 2017). Each time I visited the box, I noted whether the cups were empty or contained mealworms to track if the birds had consumed them. Nesting pairs with mealworm cups which were consistently full upon return were excluded from all analyses.

Adults were captured for measurement beginning two days after the hatching of the brood using a nest box trap (Friedman et al., 2008). At the time of capture, adults were first blood sampled, then banded, and finally, wing, tail length, mass, and tarsus were measured. We attempted to take all blood samples in under three minutes from capture. Those that took longer than three minutes to collect were excluded from CORT analysis due to a likely increase in baseline CORT because of handling stress (Romero and Reed, 2005). On day 5 post-hatch (hatch date = day 0), nestlings were individually marked with nail polish on their claws for identification and were banded with a USFWS aluminum band on day 11 post-hatch. Nestling body size was measured on days 5, 11, and 14 post-hatch. Wing length and mass were measured on all three days. Tarsus measurements were collected only on days 11 and 14. Nestling blood samples were collected on day 14. Bluebirds are sexually dimorphic as adults and nestlings (Gowaty and Plissner, 2015). Adult males have a bluer head and typically more intense coloring overall than adult females. The sex of nestlings is distinguishable by day 13 and nestlings

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with predominantly blue wing feathers were sexed as male (Gowaty and Plissner, 2015).

Further, bluebird nestlings fledge at 16-19 days (Gowaty and Plissner, 2015; Pinkowski, 1975).

Blood Sampling and Stress Physiology Methods: Blood samples (50-100 µL) were collected from all nestlings and adults within three minutes of removing from the box to accurately determine baseline CORT levels (Owen, 2011). All blood samples were taken between 0700-1100 hours to minimize variation due to circadian rhythms in hormone levels (Romero and Remage-Healey, 2000). Approximately 5-10 µl of each whole blood sample was used to prepare blood smears in the field. The remainder of each whole blood sample was kept cool on ice until it was brought to the laboratory for processing within 4 hours of collection. Blood samples were spun down for 7 minutes at 5000 rpm in a centrifuge and plasma was separated from red blood cells. The plasma was then stored at -20°C until it was used in bacterial killing assays (BKA) to quantify immune function or to measure CORT.

Slides were stained with Differential Quik Stain following kit instructions (Triangle Biomedical Sciences; Davis et al., 2008). Heterophil to lymphocyte (H:L) ratios were calculated by counting all heterophils and lymphocytes on the slide until 100 cells had been counted. Slides were not included in statistical analyses if more than 50% of the white blood cells in the inner regions of the slide appeared ruptured. Otherwise, all smears collected for each adult were counted. One nestling from each breeding attempt was selected at random for analysis. Heterophils, lymphocytes, and other white blood cells were identified by comparing them to images in the Atlas of Clinical Avian

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Hematology (Clark et al., 2009) by an expert in white blood cell identification, Ian Kanda, who was blind to the identity of the treatment groups.

Enzyme immunoassays (Corticosterone ELISA Kit ADI-901-097, Enzo Life Sciences) were used to quantify circulating CORT levels. Based on previous optimization of the assay for Eastern Bluebirds (Ambardar unpubl. data), plasma samples were diluted 1:40 and 1.5% steroid displacement reagent (SDR) was used. All samples were run in duplicate and the corticosterone standards (20,000, 4,000, 800, 500, 160, and 32 pg/mL) were run in triplicate. The optical density of the plates was then read at 405 nm on a BioTek ELx808 microplate reader. Intra-assay coefficients of variation (CV) were calculated by determining the standard deviation of sample duplicates, dividing by the mean of the duplicates, and multiplying by 100%. Samples from 2015 and 2016 were distributed across 22 assays. Samples with CVs higher than 15% were re-run once. Those that were still above 15% were removed from analyses (intra-assay CV: 6.25%). Inter-assay CVs were calculated by plating high and low concentration standards on multiple plates. The CVs for the high and low standards were calculated by finding the standard deviation of the plate triplicates, dividing by the mean of the triplicates, then multiplying by 100%. The CVs for the high and low standards were then averaged to determine the inter-assay CV (inter-assay CV: 14.57%).

Bacterial killing ability was quantified for all adults and for one randomly selected nestling within each brood. Tryptic soy agar (Fisher Scientific, DF0369-17-6) plates were made under sterile conditions and stored at 4°C the day prior to conducting assays. *Escherichia coli* (ATCC #8739, Microbiologics) stocks and dilutions were prepared the day before assays were conducted. *E. coli* stock solutions were prepared by

adding one lyophilized bacteria pellet (5.6 x 10^7 CFU) to 40 mL of phosphate-buffered saline (Sigma Aldrich, P-5368), which was then incubated at 37°C for 30 minutes and stored at 4°C. Plasma and bacteria were incubated in carbon dioxide independent media (Invitrogen Inc, Gibco media #18045). Additionally, 200 µL of 200 mM L-glutamine (Life Technologies, 25030-149) was added to the solution prior to incubation. To perform the assay, 5 µL of plasma was combined with 100 µL of the media solution and 10 µL of bacterial working solution. The bacteria and plasma were incubated together for 30 minutes at 41°C, the samples were then plated on agar plates and incubated for a minimum of 12 hours at 37°C. Control plates were prepared in the same manner as experimental plates; however, control plates did not contain plasma. The number of colonies on each plate was counted after the 12-hour incubation. The bacterial killing ability of each plasma sample was quantified as the percent difference in the number of colonies on plasma treated plates compared to the number of colonies on control plates.

Statistical Methods:

Baseline corticosterone levels were evaluated for a total of 202 individuals in the 2015 and 2016 field seasons. CORT data were analyzed using general linear mixed models with SAS 9.4 software. CORT data were not normally distributed; therefore, the data were normalized to reduce positive skew by $\log_{10}$ transforming (Lobato et al., 2009). To account for repeated measures of adult CORT levels, I included individual band number as a random effect in general linear mixed models. To account for the non-independence of young within a brood, I included nest identity as a random effect in general linear mixed models.

Analyses for BKA and H:L were performed using IBM SPSS Statistics 23 software. H:L ratio data (n = 105) were not normally distributed; therefore, I normalized the data to reduce positive skew by $\log_{10}$ transforming. H:L ratios were not statistically different between years ($F_{1,\,104}$ = 0.94, p = 0.33; 2015: mean±SE=0.73±0.09, n = 46; 2016: mean±SE=1.08±0.30, n = 59), sexes ($F_{1,\,103}$ = 0.70, p = 0.41; Female: mean±SE=1.05±0.28, n = 63; Male: mean±SE=0.74±0.11, n = 41), or age groups ($F_{1,\,104}$ = 0.17, p = 0.68; Nestlings: mean±SE=0.86±0.10, n = 55; Adults: mean±SE=1.00±0.35, n = 50). H:L ratios also were not correlated with mass, CORT, BKA, brood size, or collection date (all p > 0.60); therefore, a one-way analysis of variance (ANOVA) with food treatment as the predictor variable was used and data were analyzed independent of sex, age, or year of sampling.

A total of 118 individuals were evaluated for bacterial killing ability in the 2015 (n = 46) and 2016 (n = 72) field seasons. To assess normality, I inspected normality curves and skewness and kurtosis values. BKA was analyzed using a one-way ANOVA. I first tested if the fixed effects of year, sex, and age class significantly influenced BKA. Bacterial killing ability was significantly lower in nestlings than in adults ($F_{1,\,116}$ = 18.68, p < 0.001; adults: mean±SE=55.63±3.89%, n = 50; nestlings: mean±SE=36.28±2.56%, n = 68; Fig. 1). As a consequence, data for adults and nestlings were analyzed separately. Within the adults, there was no significant difference in bacteria killing ability between males (mean±SE=52.19±5.83%, n = 21) and females (mean±SE=58.76±5.40%, n = 28; $F_{1,\,47}$ = 0.67, p = 0.42). BKA quantified in adults also was not significantly impacted by year ($F_{1,\,47}$ = 0.18, p = 0.68; 2015: mean±SE=53.83±6.75%, n = 19; 2016: mean±SE=57.28±4.92%, n = 30). Within nestlings, there also was no significant

difference in mean bacteria killing ability between males (mean±SE=34.56±4.67%, n = 31), and females (mean±SE=37.79±2.60%, n = 38; $F_{1, 67}$ = 0.40, p = 0.53). Among nestlings, there also was no significant difference in mean bacteria killing ability between samples collected in 2015 (mean±SE= 35.11±5.28%, n = 27) and those collected in 2016 (mean±SE=37.13±2.45%, n = 42; $F_{1, 67}$ = 0.15, p = 0.70).

RESULTS

Baseline Corticosterone: In adults, CORT concentrations tended to be negatively correlated with body mass (r = -0.26, d.f. = 56, p = 0.06); therefore, mass was included as a covariate in adult CORT models. There was no significant effect of supplement group on baseline corticosterone concentrations ($F_{2, 26.1}$ = 1.34, p = 0.28; control: 10.20±1.19 ng/mL; part: 9.21±1.10; full: 12.00±1.53 ng/mL; Fig. 2). Similarly, there was no significant effect of supplement group on baseline corticosterone levels in nestlings ($F_{2, 64.5}$ = 0.45, p = 0.64; control: 8.57±0.67; part; 9.77±1.21; full: 9.26±0.88 ng/mL; Fig. 3).

Heterophil to Lymphocyte Ratios: There was no significant effect of supplement group on heterophil to lymphocyte ratios ($F_{2, 101}$ = 0.17, p = 0.85; control: 0.54±0.32, n = 55; part: 0.56±0.13, n = 24; full: 0.62±0.13 n = 23; Fig. 4).

Bacterial Killing Ability:

In the adults, no significant correlations were found between bacteria killing ability and brood size (r = 0.10, n = 49, p = 0.51) or date of blood sample collection (r = 0.04, n = 49, p = 0.81). In adults, BKA was negatively correlated with body mass (r = -0.29, n = 49, p = 0.04). Thus, mass was included as a covariate in the final univariate ANCOVA model. There was no significant difference in bacteria killing ability among

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the control (mean±SE=57.53±5.43%, n = 21), part (mean±SE=56.14±6.42%, n = 14), and full treatment groups (mean±SE=53.36±9.60%, n = 14; $F_{2, 49} = 0.36$, p = 0.70; Fig. 5).

No significant relationships were found between nestling bacteria killing ability and brood size (r = -0.03, n = 69, p = 0.80) or date of blood sampling (r = 0.01, n = 69, p = 0.91). Next, I tested if BKA was significantly correlated with body mass. In nestlings, bacterial killing ability was positively correlated with body mass (r = 0.35, n = 69, p = 0.004). Thus, I included body mass as a covariate in ANCOVAs to analyze differences in BKA among the experimental groups. In nestlings, there were no significant differences in bacterial killing ability among the control (mean±SE=38.64±3.83%, n = 32), part (mean±SE=29.26±5.81%, n = 14), and full treatment groups (mean±SE=37.44±4.07% n = 23; $F_{2, 69} = 0.94$, p = 0.40; Fig. 6).

DISCUSSION

I predicted that corticosterone (CORT) levels and heterophil to lymphocyte ratios (H:L) would be highest in adults and nestlings in the PART group due to this group facing the greatest potential food strain compared to the CONT and FULL groups (PART>CONT>FULL). My results indicate that supplemental feeding did not substantially influence baseline CORT or heterophil to lymphocyte ratios. I also predicted that bacterial killing ability would be highest in the FULL group (FULL>PART/CONT). My analyses showed there to be similar bacterial killing ability across trial groups. Therefore, my supplemental feeding groups did not appear to influence stress physiology or immune function.

Possible reasons for these results include that CORT has multiple modes of activation and is sensitive to changes in weather events amongst other environmental changes such as pollution and increased predation (Martin, 2009; O'Neal and Ketterson, 2011; Wingfield, 1995). Because I conducted the manipulation of supplemental feeding in the field, it is possible other changes in the environment may have had an influence on CORT concentrations. Heterophil to lymphocyte ratio may also have been influenced by environmental changes as this metric is modulated by CORT (Davis et al., 2008; Dhabhar et al., 2009). However, there was no significant correlation between H:L and CORT in my study, as has been observed in previous research and similar H:L ratios were seen across supplement groups. An area for future research would be to evaluate the effects of weather changes and severe weather events across the season on baseline corticosterone levels and H:L.

In contrast to many other studies in which supplemental food was manipulated, my experimental design did not increase exposure to disease, a potential consequence of congregation around feeders, as all of the mealworms were distributed inside the nest boxes (Robb et al., 2008b). This design was utilized both to: 1) insure that the birds breeding in the nest box were the only ones who could access the food and 2) isolate the effects of food supplementation from any effects of increased disease exposure on immune function and stress metrics. This is a major difference from previous studies in which feeders were mounted externally (Brittingham and Temple, 1986; Wilcoxen et al., 2015).

In adults, males and females had similar bactericidal capabilities. Investment in immune function may be sexually dimorphic with males typically being

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immunosuppressed relative to females as a trade-off for reproductive investment (Klein, 2000; Zuk, 1990). Baseline CORT was nearly negatively correlated with mass in adults, which is similar to previous results from other species. Previous studies have found a stronger negative relationship between corticosterone and body mass in adults, and suggest this is the result of breeding-induced trade-offs in adults as adult birds may reduce body mass when food conditions are poor to better care for nestlings (Jenni-Eiermann et al., 2007). This may have occurred in the bluebirds as well. Hormonal stress response is also dependent on physical condition of birds (Wingfield, 1995). In arctic birds, individuals with larger fat stores were better adapted to the increased stress of winter storm conditions allowing heavier birds to maintain homeostasis without increasing CORT secretion (Wingfield, 1995). Passerines do not commonly store fat on their bodies during the breeding season; therefore, I did not evaluate existing fat stores in adult bluebirds. However, it would be of interest to evaluate parental condition prior to the breeding season as this may influence the ability of adults to care for nestlings.

Nestlings have been shown to have lower bactericidal function than adults due to their reduced ability to synthesize antibodies (O'Neal and Ketterson, 2011). Maternal condition prior to egg laying may influence both nestling hormone levels and immune function. Nestling immune function is also influenced by hormones and antibodies deposited in the egg during yolk production (Boulinier and Staszewski, 2008; Grindstaff et al., 2003). I manipulated food availability over a few days immediately prior to laying, so there may have been an effect on the last laid eggs of clutches during my experiment. However, I did not distinguish between first and last eggs laid and these potential differences were not accounted for when comparing supplement groups. Measuring the

relationship between laying sequence and immune function would be of interest for future studies. Further, the addition of food supplement for a longer period of time and in higher quantities prior to egg laying could potentially cause greater variation in immune function.

Overall, supplemental feeding did not significantly influence baseline corticosterone, bactericidal capacity, or heterophil to lymphocyte ratios in nestlings or adults. This does not support my predictions that: 1) nestlings would have increased CORT and H:L in the PART group and 2) nest boxes provided with supplemental food throughout the nesting attempt would have increased BKA. Another potential reason for the non-significant effect of food supplementation is that the experimental manipulation may not have substantially impacted the birds' natural foraging behavior. When bluebirds are feeding nestlings, 100 mealworms provided twice daily is considered sufficient to sustain both the adults and nestlings in one nest box (Smith, 2017; NABS, 2017). Dependent on the number of nestlings present, I provided 45-120 mealworms in each box once a day, three days a week. This amount of supplementation may have been insubstantial compared to the twice daily supplementation recommended by Smith (2017). This supplementation approach was taken due to the scale of my study which involved travelling to numerous boxes at different sites, as compared to the backyard feeders for which Smith (2017) and NABS (2017) make their recommendations. Further, as seen in previous studies, an abundance of food sources reduces potential food stress to the birds and presumably increases survival, but additional food is only beneficial when there is a lack of food in the environment (e.g., Schoech et al. 2007).

A study conducted by Willson and Harmeson (1973) found that handling time of the food source was the most significant driver of seed preference in Song Sparrows (*Melospiza melodia*) and Northern Cardinals (*Cardinalis cardinalis)*. Eastern Bluebirds are predominantly insectivorous so seed cracking is not relevant to this species, but it remains possible that handling time may have affected the birds' ability to use the mealworms in the boxes. It was not uncommon to find mealworms in the nesting material after the nestlings fledged and the old nest material was removed. The mealworms were alive when placed in feeding cups, though not capable of climbing out of the feeding cups. The loose mealworms in the nesting material may indicate the adults struggled to pick up and immobilize the mealworms while they were in the nest box. However, the adults were documented using the mealworms in nest boxes through the use of next box cameras (2 of approximately 70 observations; Pandit, unpublished data). Further, the number of mealworms that were found in the nesting material was substantially less than the number of mealworms provided over the course of the nesting cycle, so I am confident the bluebirds consumed the majority of the mealworms (Perryman, personal observation).

Based on the results of previous studies and the current study, it would be beneficial to comprehensively evaluate natural food availability in relation to manipulation of supplemental food availability (Fay, 2003). It would also be beneficial to evaluate differences in food quality to determine if differences in nutritional content between mealworms and natural food sources may have impacted my results. Zebra finches (*Taeniopygia guttata*) with access to protein supplementation laid heavier eggs than females without supplementation (Williams, 1996). Mealworms are low in calcium,

which aids in appropriate bone development and egg production (Finke, 2002). Therefore, if a bird were exclusively feeding on mealworms this could be detrimental to individual bone strength and reproductive success.

Differences in nutritional quality may cause Eastern Bluebirds to exhibit a preference for other invertebrates over mealworms. With the possible exception of winter when natural food is scarce (Robb et al., 2008a), birds prefer natural food sources over supplemental sources. In a study by Brittingham and Temple (1988), black-capped chickadees (*Parus atricapillus*) were provided with supplemental feeders in the winter. Even during the winter period, chickadees only supplemented their diet with the food source 21% of the time. The remainder of their diet was gathered from natural foraging. This result suggests that foraging may be beneficial to birds beyond simply the need to attain food. Further, supplemental feeding during times of food abundance may provide only a small subsidy of the large amount of food needed to survive or raise a brood. It would be of interest to conduct this study during the winter to potentially see a greater influence of supplemental feeding on immune function and stress physiology metrics in adults.

While I did not find evidence that supplemental feeding benefitted bluebirds, there also was no detrimental effect. Supplemental feeding is a pastime that could have a net beneficial effect for birds given the side benefit of engaging the public in the outdoors (Newsome and Rodger, 2008). As I observed while conducting this study, nearby citizens eagerly await the opportunity to get involved in science and, the use of citizens for data collection is increasingly common. An early collaboration of amateur scientists on a broad scale, begun in 1749, has been essential to the collection of bird migration data in

several European countries and is still being conducted today (Dickinson et al., 2010; Greenwood, 2007). Conversely, non-collaborative studies conducted by a single researcher have limited potential for longevity as such studies may be limited by the career span of the individual researcher. Collaborative science initiatives are additionally beneficial because citizen science projects can help establish baseline data for additional research (Dickinson et al., 2012). Finally, the increased public understanding that comes with citizen participation in science can increase public support for future scientific studies and creates a sense of environmental stewardship (Dickinson et al., 2012).

It would be of interest in the future to set-up feeders in nest boxes at citizen's houses within my study area. This could allow for a longer term study and the evaluation of how supplemental feeding affects birds living in a suburban area. Further, as the focal human-wildlife interaction in my study, it would be very beneficial to simulate this interaction as closely as possible. This would be critical to understanding a common human-wildlife interaction and how it affects health and productivity of bird species.

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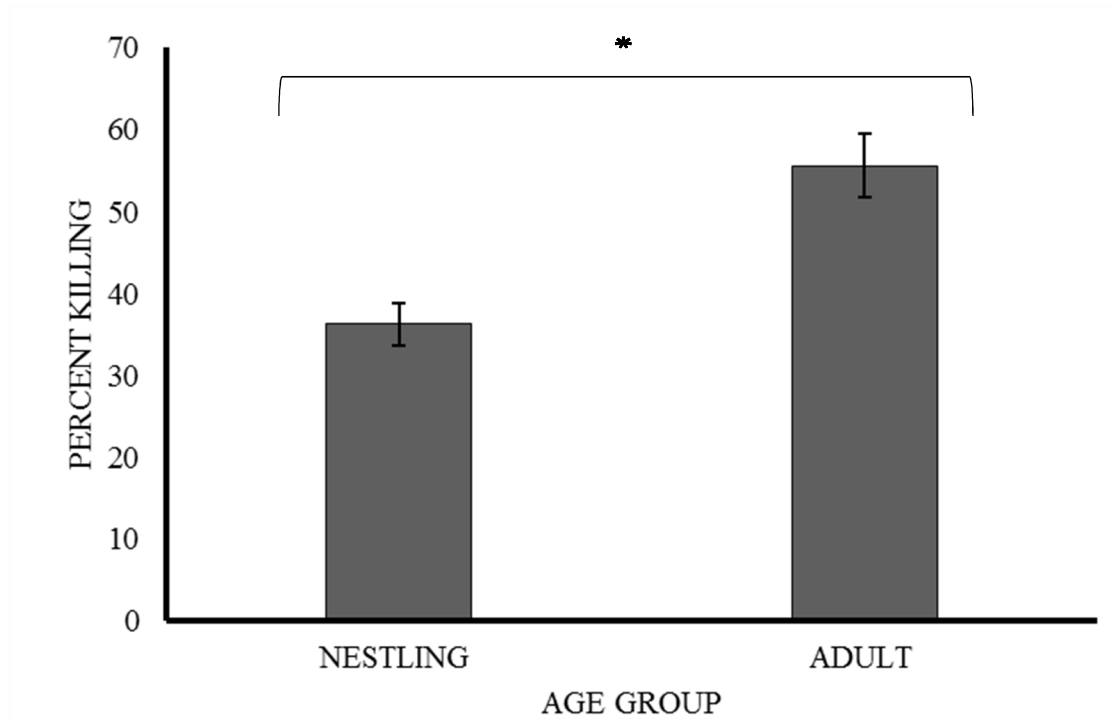
Figure 1. Mean percent bacterial killing ability in nestlings and adults across feeding groups. Error bars depict calculated standard error. Significant differences (at p < 0.05) indicated by asterisk.

Figure 2. Mean corticosterone concentration in ng/mL in blood plasma of adults in the control, partially supplemented, and fully supplemented groups. Standard errors are indicated by error bars on the chart
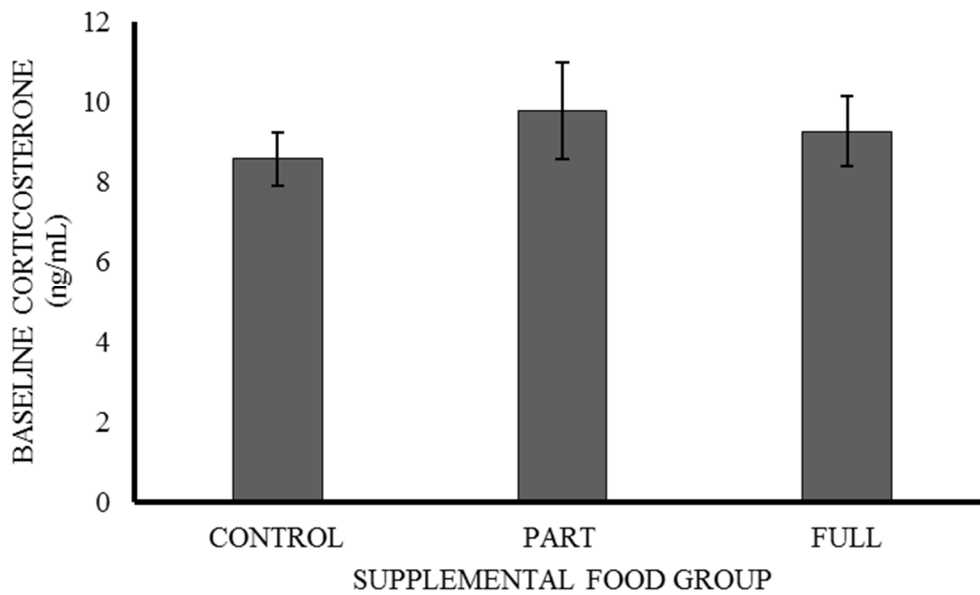
Figure 3. Mean corticosterone concentration in ng/mL in nestling blood plasma in the control, partially supplemented, and fully supplemented groups. Standard errors are indicated by error bars on the chart

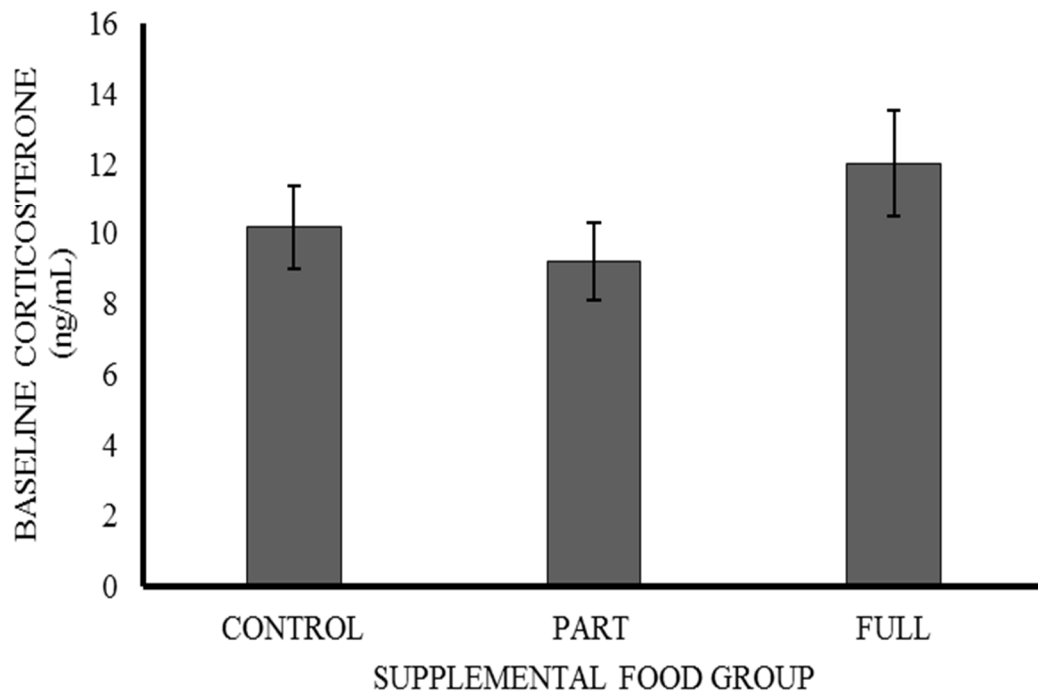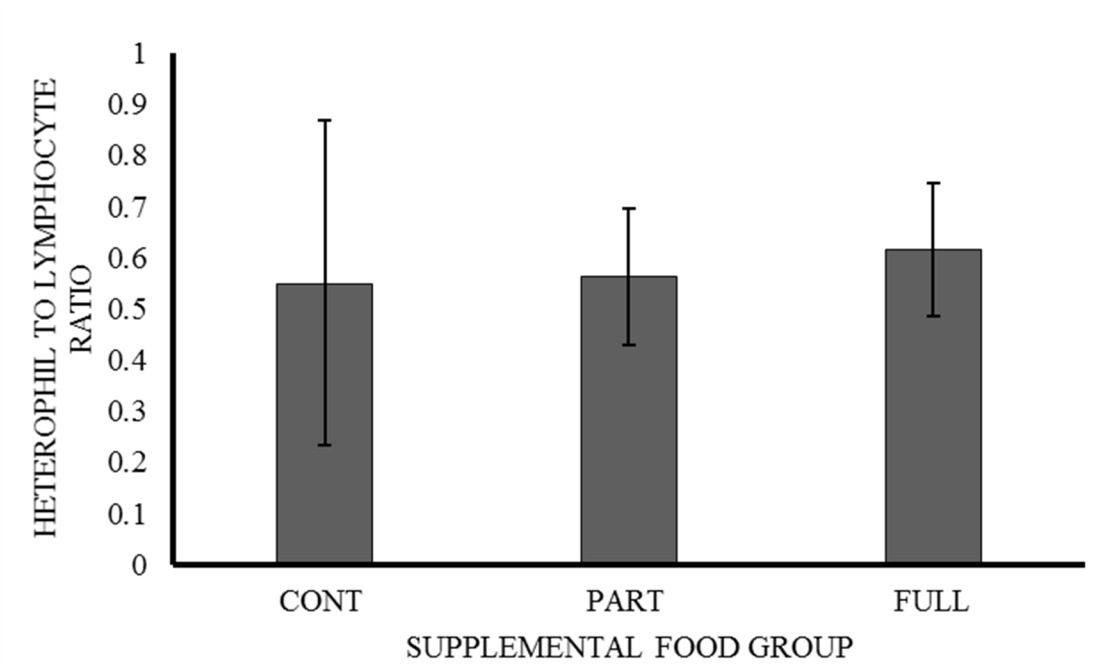*Figure 4. Mean heterophil to lymphocyte ratio across feeding groups (non-transformed). Error bars depict calculated standard error*

Figure 5. Mean percent bacterial killing ability in adults from control, partially food supplemented, and fully supplemented groups. Error bars depict calculated standard error of each group

Figure 6. Mean percent bacterial killing ability in nestlings from the control, partially food supplemented, and fully supplemented groups. Error bars depict calculated standard error of each group
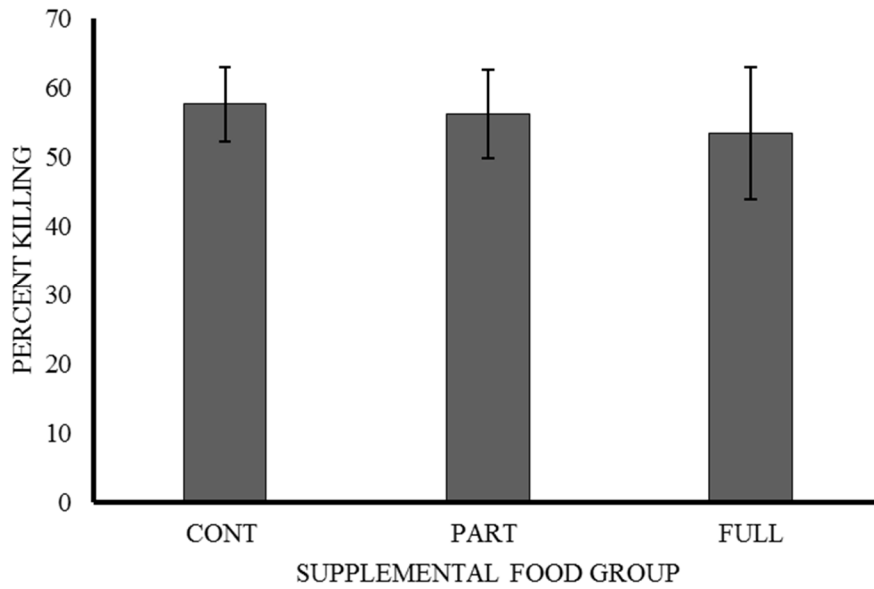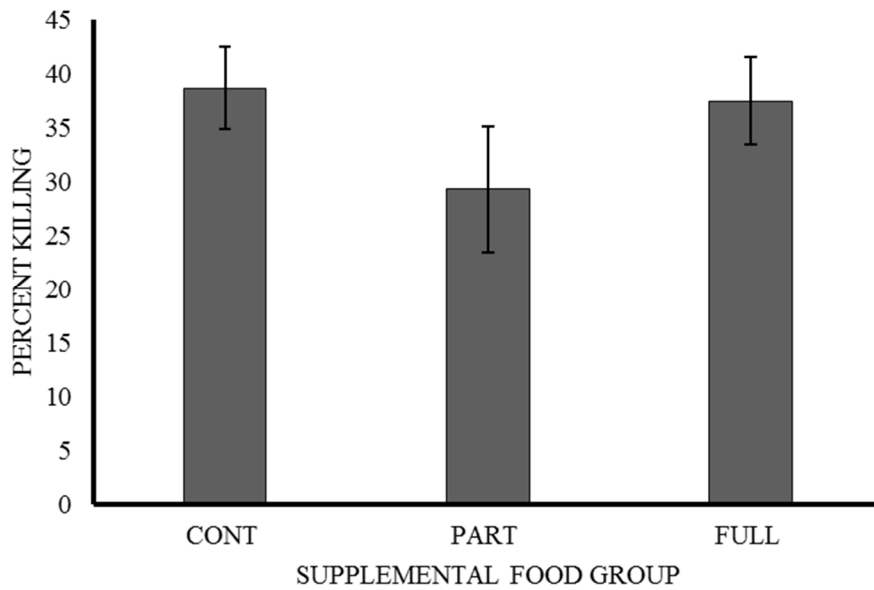
CHAPTER THREE

EFFECTS OF SUPPLEMENTAL FEEDING ON EASTERN BLUEBIRD NESTING

SUCCESS

INTRODUCTION

While humans have well-documented negative impacts on wildlife populations,

certain anthropogenic effects may be beneficial to wildlife. A potential positive

anthropogenic effect on avian nesting success is supplemental feeding (Marzluff, 2001).

Supplemental, or supplementary, feeding is the addition of food sources, typically

anthropogenic, to a species' diet beyond that which is naturally available (Robb et al.,

2008a; Sguassero et al., 2012). This additional food source can be added deliberately, as

is the case for residential bird feeders, or incidentally as in the case of an open waste

disposal area. For songbirds, backyard bird feeders may be an important source of

supplemental food in urban and suburban environments (Meillère et.al, 2015). Despite

the prevalence of backyard birdfeeding, comparatively few studies have experimentally

manipulated food supplementation and examined its influence on nesting success.

Previous studies have researched how existing feeders influence nesting success and how

increased urbanization influences natural food availability (Meillère et al., 2015;

Tryjanowski et al., 2015).

Some of the most extensive previous research on the effects of supplemental feeding on reproductive success was conducted on Florida Scrub Jays (*Aphelocoma coerulescens*). Scrub jays with constant access to supplemental food sources generally laid eggs earlier in the season than non-supplemented birds, especially when natural food availability was low (Schoech et al., 2009). Fleischer et al. (2003) studied two populations of scrub jays which differed in the timing of breeding and also bred in habitats with differing degrees of urbanization. Birds in the more urbanized habitat had increased access to human food sources including cracked corn, bird seed, and food provided for domesticated animals. Thus, it was concluded that supplemental food can advance laying date and cause changes in foraging efficiency (Fleischer et al., 2003).

The impact of supplemental feeding on the survival of Black-capped Chickadees (*Parus attricapillus*) was evaluated by concurrently studying supplemented and unsupplemented birds (Brittingham and Temple, 1986). This study was one of the first to include a control for annual variation in natural food availability. The study used comparable sites randomly designated as experimentally supplemented or controls. Experimentally supplemented sites were provided with feeders during the winter, with the intent to evaluate subsequent breeding success. During the summer months, when the birds were no longer receiving supplementation, there was no difference in the survival rates of chickadees between the experimental and control sites, but it was also observed that birds only foraged at the feeders 21% of the time in winter (Brittingham and Temple, 1986).

A study on the effects of supplemental feeding on Red-Winged Blackbirds (*Agelaius phoeniceus*) included more bird feeders in the study site and evaluated the protein content

of the supplemental food. Although supplemental food sources advanced the laying dates of blackbirds, predation events and both intra- and interspecific competition increased around the feeders (Ewald and Rohwer, 1982). This demonstrates the limits of the beneficial effects of supplemental food.

Despite providing an additional food source for birds, there are also potential negative impacts of supplemental feeding on birds. Bird feeding may increase bird population sizes beyond numbers that are sustainable by the environment once the supplemental food source is removed (Robb et al., 2008b). Also, bird populations have been shown to benefit initially from supplemental food, but later suffer increased mortality due to a loss of synchrony with natural food availability (Schoech and Bowman, 2001). Birds generally breed when natural food sources are most abundant, which for an insectivorous and invertebrate consuming species, such as the Eastern Bluebird (*Sialia sialis*), is in the spring and summer (Gowaty and Plissner, 2015). Supplemental feeding can simulate peak food abundance at a time of year that is not optimal for rearing a brood; therefore, failure to maintain the supplemental food source may negatively affect nestling survival (Robb et al., 2008b). Further, it is important to evaluate natural food availability to determine if food is limited for the species at the time of the study.

It is possible supplemental feeding may be provided inconsistently through variable provisioning at an established feeder (Wilcoxen et al., 2015). In such conditions, birds at supplemental feeders may be subject to removal of a significant food source during critical times in the breeding cycle. Few studies have examined how removal of a supplemental food source during the nestling stage may impact nest success (Brittingham and Temple, 1988; Harrison et al., 2010). Addition and then subsequent removal of a

supplemental food source at a critial time point in the breeding cycle, such as hatching, could cause adults to initially increase parental investment (e.g., by increasing clutch size) without sufficient resources or time to devote to the investment if the supplemental food source is removed. It is important to understand how these manipulations of food availability could affect bird productivity, which is important for best managing populations in a human dominated landscape.

In this study, I manipulated supplemental food availability to Eastern Bluebirds, a species which is commonly a target of supplemental feeding. Eastern Bluebirds are a secondary cavity nesting species with a native range that spans from the central United States to the Atlantic Coast (Sibley, 2014). Bluebirds often use nest boxes as breeding sites (Gowaty and Plissner, 2015). This species is ideal for this study as, given their dependence on secondary cavities for nesting, it is possible to easily locate nests and manipulate food availability. Birdwatchers commonly provide bluebirds with mealworms (*Tenebrionidae* beetle larvae) as a supplemental food source (Smith, 2017). Bluebirds breed up to three times per season in Oklahoma, laying clutches of 3-5 eggs each time (Gowaty and Plissner, 2015). To determine the effect the removal of a supplemental food source during the nesting cycle has on nestling growth and nest success, I included two groups that were provided with supplemental food; however, in one group the supplemental food source was removed when young hatched. The first of my two experimental trials received supplementation from the nest completion stage until fledging (FULL), and the second group was given supplementation from the nest completion stage until hatching (PART). The control group (CONT) was not given supplementation at any point. All boxes were opened the same number of times

regardless of experimental trial. I expected nesting success of birds in the FULL group to be greater than nesting success of birds in the PART group or the CONT group. I also conducted invertebrate abundance surveys to approximate natural food availability in the environment and to determine the effect of natural food availability on the response to a supplemental food source.

METHODS

Nest box monitoring: Wooden nest boxes (n = 175) were mounted along trails in Stillwater, Oklahoma (36°7′18″N 97°4′7″W). Nest boxes were monitored from March-August in both 2015 and 2016. Each nest box was checked 2-3 times per week throughout the breeding season. When completed nests were discovered, the box was then monitored daily for egg laying. Daily monitoring occurred again when clutches neared their expected hatching date (clutch completion date + 13 days) to accurately determine hatch date. Finally, broods were monitored daily after day 14 to determine approximate fledging date and fledging success. Clutch size, hatching success, nestling growth, and fledging success were recorded at each of the nest boxes in all three treatment groups. Clutch size was quantified as the number of eggs laid in the nest. A clutch was considered complete when the number of eggs did not increase for more than one day. Hatching success was quantified as the proportion of eggs in the clutch that hatched. Brood size was quantified as the number of eggs that hatched in the nest. Fledging success was determined as the proportion of nestlings that left the nest after the day 14 measurement. The standard time to fledging in Eastern Bluebirds is 17-19 days (Gowaty and Plissner, 2015) with nestlings capable of weak flight at day 14. Nestlings absent from the nest after day 14 were considered successfully fledged, unless there was

evidence of death or predation. Examples of evidence for death or predation included a dead bird in or near the box, predator presence inside the box, or evidence of predation such as an excess of feathers on the ground near the box.

Experimental manipulation of food availability: To provide supplemental food, small plastic cups (2 oz., clear) were mounted inside nest boxes in February prior to the start of each breeding season. Nest boxes assigned to receive supplemental food were provided with fifteen mealworms (1.909 ± 0.177g) per individual bluebird in the nest box, including both nestlings and adults, three times weekly (Smith, 2017). The first experimental group was the fully supplemented group (FULL; 2015: n = 35, 2016: n = 22) which received mealworms from nest building through the remainder of the breeding attempt. The partially supplemented group (PART; 2015: n = 26, 2016: n = 20) received mealworms from nest construction until hatching. The control group (CONT; 2015: n = 36, 2016: n = 35) did not receive mealworms at any point. All boxes, regardless of experimental treatment, were visited and opened the same number of times to control for any effects of disturbance.

Capture: All birds were captured to collect morphological measurements. Adults were captured with a nest box trap no sooner than the second day after hatching. Adults were measured at the time of capture. Mass for each individual was determined using either a digital balance (accuracy =0.01 g) or Pesola (accuracy=0.1 g). Wing chord and tail length of the two outermost retrices were measured using a wing rule (accuracy=1 mm). Tarsus length was measured using calipers (accuracy = 0.01 mm). Nestlings were measured on days 5, 11, and 14 post-hatch (day 0=hatch day). Wing length and mass measurements were collected on all three of these days, while tarsus length was measured only on days

11 and 14. Toenails of nestlings were painted with nail polish on day 5 to distinguish

individuals, then on day 11, nestlings were banded with an aluminum USFWS band.

Estimates of natural food availability: Relative abundance of probable food sources for

Eastern Bluebirds (insects and other invertebrates (Gowaty and Plissner, 2015)) was

approximated using pit fall traps and sweep netting (Fay, 2003). Invertebrate surveys

(2015: n = 27 ; 2016: n = 22) were conducted in 2015 and 2016 to approximate

invertebrate order abundance and order richness near a subset of active bluebird nest

boxes. Surveys were conducted as time permitted during the breeding season at boxes

with clear evidence of activity (primarily during the nestling stage). Similar numbers of

surveys were conducted in each supplemental feeding group and on each nest box trail. A

5x5 m grid was surveyed for invertebrate abundance and richness at least 10-40 m from

selected active nest boxes to minimize disturbance (Fig. 1). When possible, surveys were

conducted at a compass heading within which the breeding pair at the nest box had been

seen previously (Fig. 2; Fay 2003). If this was not possible, a heading was randomly

selected (1-360°), then 10 m away in the direction of the heading was established as the

corner of the quadrat within the plot area (Fig. 3). When headings to potential transect

areas were on private property or were blocked by other physical barriers, a new heading

was chosen. Next, five plastic pitfall traps were buried (approximately 55 mm deep for

the cup to be flush with the ground) in the ground and distributed randomly by assigning

them a random coordinate within a quadrat. All invertebrates captured in the pitfall traps

after 24 hours were collected. In 2016 pitfall traps were instead spaced 1 m apart in a

straight line across the transect area (Fig. 4). This method was useful when accessible

space near the nest box was limited, and the likelihood that eligible areas around the nest

box would be surveyed was similar. All other survey methods were consistent between 2015 and 2016. The transect area was swept 30 times in parallel lines spaced 1 m apart in a sinusoid fashion (Fay, 2003). All of the invertebrates in the sweep net were counted at the time of the sweep. Relative invertebrate abundance (order abundance) was approximated by counting the number of invertebrates in all five of the pitfall traps and sweep net. Order abundance was considered to be the number or frequency of individuals in an order occurring in a survey area. Order richness was determined as the number of different orders caught in the pitfall traps and sweep net at each survey site. Invertebrates were identified in the field based on morphology and released. Samples collected from pitfall traps were identified in the lab based on morphology.

Invertebrates under 8 mm in length are minor food sources used by bluebirds to feed to their nestlings (3-7% of observations compared to 35-41% of observations for Lepidoptera larvae); therefore, I used 8 mm as a size cut-off for inclusion in further analyses (Gowaty and Plissner, 2015; Pinkowski, 1978). Invertebrates under the 8 mm size cut-off were not included in abundance analyses because they were very difficult to field identify to order and were difficult to collect or store from pitfall traps (Pitts, 1978).

Statistical Methods: Data on nesting success were analyzed using SAS 9.4 software. Pearson's correlations were run to determine if adult morphometric measures (wing length, tail length, mass, and tarsus length) were significantly correlated with clutch size, hatching success, brood size, or fledging success. Although morphometric measures were generally correlated with one another, adult size was not correlated with any of the measures of nesting success (all $p > 0.12$). Therefore, I did not include adult body size in the final models. I also ran correlations between nestling growth measurements and the

breeding success metrics and found no significant correlations (all $p > 0.08$). I then ran general linear models to determine if there were significant differences between 2015 and 2016 in clutch size (CS), hatching success (HS), brood size (BS), and fledging success (FS). No significant differences were found between years [(CS: $F_{1, 82} = 0.16$, $p = 0.69$); (HS: $F_{1, 82} = 0.03$, $p = 0.87$); (BS: $F_{1, 82} = 0.06$, $p = 0.81$); (FS: $F_{1, 81} = 1.31$, $p = 0.26$)]. Therefore, both years were analyzed together in subsequent analyses. I then ran linear mixed models on CS, HS, BS, and FS to determine if supplemental feeding affected any of these metrics. Female band number was included as a random effect in mixed models to account for repeated measures of some individuals.

Invertebrate abundance data were analyzed using IBM SPSS Statistics 23 software. Order abundance (OA) and order richness (OR) data were not normally distributed; therefore, I normalized the data to reduce positive skew by $\log_{10}$ transforming after adding one to eliminate zeroes. I then conducted independent samples t-tests to determine if there were any between year differences in OA (2015: mean±SE: 0.72±0.09, n = 27; 2016: mean±SE: 0.75±0.10, n = 22) and OR (2015: mean±SE: 0.47±0.05, n = 27; 2016: mean±SE: 0.49±0.06, n = 22) and found there were no significant differences between years for OA ($t_{47} = -0.21$, $p = 0.98$) or OR ($t_{47} = -0.27$, $p = 0.95$). Next, I ran Pearson's correlations to determine if nest box trail, time of sweep netting, invertebrate survey date, or average grass height were correlated with OA or OR. No significant correlations between sweep net time (OA: $r = -0.10$, $p = 0.62$; OR: $r = 0.02$, $p = 0.90$), survey date (OA: $r = -0.11$, $p = 0.49$; OR: $r = -0.08$, $p = 0.60$), or grass height (OA: $r = 0.29$, $p = 0.16$; OR: $r = 0.33$, $p = 0.10$) were found. I then conducted correlations to determine if order abundance and richness were correlated with CS, HS, BS, or FS.

Finally, I ran ANCOVA models with OA or OR as covariates and supplemental feeding group as a fixed effect to determine if natural food availability influenced the relationship between supplemental feeding and nest success.

RESULTS

Clutch size was not significantly affected by supplemental feeding (mean±SE: control: 4.47±0.13; part: 4.73±0.17; full: 4.64±0.16; $F_{2, 72.3}$ = 0.76, p = 0 .47; Fig. 5). Hatching success also was not significantly influenced by supplement group (mean±SE: control: 0.85±0.04; part: 0.88±0.05; full: 0.76±0.05; $F_{2, 72.6}$ = 1.84, p = 0.17; Fig. 6). Brood size was not significantly influenced by supplement group (mean±SE: control: 3.72±0.19; part: 4.15±0.24; full: 3.54±0.22; $F_{2, 75.5}$= 1.93, p = 0.15; Fig. 7). Finally, fledging success also was not significantly influenced by supplemental feeding ((control (mean±SE: 0.91±0.05), part (mean±SE: 0.76±0.07), full (mean±SE: 0.84±0.07; ($F_{2, 69.9}$ = 1.55, p = 0.22; Fig. 8)).

Observations of invertebrate order abundance at the study sites are detailed in Table 1. Order abundance (OA) and order richness (OR) were not correlated with any of the nesting success metrics (OA: CS, HS, BS, FS, all p > 0.35; OR: CS, HS, BS, FS, all p >0.32). Finally, I ran ANCOVA models to determine if there were significant interactions between supplemental feeding group and invertebrate data as it related to the four breeding success metrics. There was no relationship seen for CS ($F_{2, 17}$= 0.25, p = 0.79), HS ($F_{2, 17}$= 1.18, p = 0.34), or BS ($F_{2, 17}$= 0.68, p = 0.52). FS did not meet the assumptions necessary to run an ANCOVA due to low variability in the sub-sample of nest boxes at which I conducted invertebrate surveys.

DISCUSSION

My hypothesis that access to supplemental food would increase clutch size, hatching success, brood size, and fledging success was not supported in my study as none of these metrics were significantly influenced by food supplementation. A similar study conducted by Wilcoxen et al. (2015) evaluated the effects of supplemental feeding on a variety of seed eating birds. These authors quantified, among other metrics, body condition, feather quality, anti-oxidant levels, and reproductive physiology. They saw some improvement in health of individuals using supplemental feeders including faster feather growth and increased anti-oxidant levels. However, birds in areas with supplemental feeding were also more likely to be infected with diseases, including conjunctivitis, avian pox, dermal disease or cloacal disease, and infected birds were not in better physiological condition (Wilcoxen et al. 2015). One benefit of the method I used for supplemental feeding is that it did not cause birds to aggregate and increase exposure to disease. Supplemental food provided away from nest sites may provide a better representation of normal foraging behavior, but does not easily allow researchers to track the amount of supplemental food consumed or to track the individuals utilizing the food source. Additionally, an excess of natural food availability could reduce the birds' dependence on supplemental food sources (Schoech et al., 2007). It is important then to understand how natural food availability may influence the response to supplemental food.

Although I did not detect effects of natural food availability on nest success metrics, it is still possible natural food abundance may have an influence on the effectiveness of supplemental feeding. Utilizing a similar experimental design, Rooney

and colleagues (2014) manipulated diet in a bird of prey, Common Buzzards (*Buteo buteo*). A supplemental food source was provided to the buzzards and consumption of the food was closely monitored. Buzzards were provided food in one of three experimental groups: 1) prior to laying, 2) post-laying, or 3) throughout the breeding cycle. There also was a control group that received no supplementation. Additionally, habitat composition and quality were evaluated using analysis of land cover data in ArcGIS. The study found that habitat quality had the largest influence on breeding success in buzzards, and that supplemental feeding had the most prominent effect on individuals in low quality habitats. In my study, use of land cover data, specifically comparing differing types of grass cover, may be a useful metric for evaluating bluebird habitat quality.

In Oystercatchers (*Haematopus ostralegus*), and Curlews (*Numenius arquata*), prey capture rates increased in association with human disturbance. This could indicate some species are able to forage quickly in anticipation of interruptions in foraging (Fitzpatrick and Bouchez, 1998). In the PART group in my study, I maintained disturbance while removing a food source and potentially interrupting foraging time on the territory. It is possible the birds increased foraging prior to or after my disturbing them, and were able to compensate for the removal of the food source in this group. Additionally, I conducted my invertebrate surveys only around active nest boxes, particularly those with eggs and nestlings. At this point in the nesting cycle, the pair may have already selected the territory by evaluating natural food availability and other resources that influence food availability and reproductive potential (Cody, 1981). As a consequence, active nests were most likely located on territories with sufficient natural food availability. Further, I surveyed a relatively small portion of the nesting pairs'

territories, which can span 1-8 hectare (Gowaty and Plissner, 2015; Kreig, 1971). It is possible that the invertebrate surveys did not fully reflect the food sources available to bluebirds through foraging. The surveys also were not conducted at controlled stages of the breeding cycle, instead varying somewhat across sites. This could contribute to the lack of interaction between the supplement groups and order abundance or richness.

I did not detect any significant benefits or costs of supplemental feeding of Eastern Bluebirds. It is possible the birds remained reliant on natural food sources. From nest box camera observations, bluebirds were seen feeding their nestlings mealworms on two out of nearly 70 observations (Pandit, unpublished data). However, it is still likely the birds with empty feeding cups upon return consumed the mealworms as: 1) some birds consistently had full mealworm cups when I returned the next time to feed mealworms and did not actively remove the worms and 2) birds were never independently observed removing the mealworms from the box during video observations. I noted whether cups were full, had some left, or were completely empty during my visits to provide mealworms. Any nesting pairs with consistently full cups were removed from all analyses. There was no evidence that the birds removed the mealworms to maintain nest cleanliness. Therefore, the idea that the birds removed the mealworms as a nest-fouling object (similar to fecal sacs) was rejected. This supports the idea that the birds ate the mealworms when the cups were empty.

Individual preference may also influence foraging. Hummingbirds have been observed to prefer natural food sources, unless flowering plants and nectar availability are scarce, even though supplemental nectar feeders were easily accessible (McCaffery and Wethington, 2008). Natural food sources may have greater nutritional content than

supplemental food sources (McCaffery and Wethington, 2008). In my study, some pairs did not consume mealworms in the feeding cups, despite ease of access [6/63 (9.53%) boxes total for both years which received PART/FULL treatments and survived to day 5]. Future studies should utilize calorimetry analysis of food sources in the environment and the supplemental food, and analyze protein and calcium content. Further, designing a study that accounts for food preferences of birds would be valuable.

The primary goal of this project was to determine whether providing mealworms as a supplemental food source to Eastern Bluebirds has direct benefits for bluebirds. Although I did not find direct benefits of supplemental feeding for bluebirds, bird feeding can be beneficial to the community, professional scientists and wildlife in general (Newsome and Rodger, 2008). Through scheduled interactive feeding activities at recreational sites, the community can attract tourism to an area, which can in turn increase revenue for the recreation site to be used for conservation purposes, so there is the possibility that any detrimental effects of supplemental feeding will be outweighed by these beneficial effects (Newsome and Rodger, 2008; Horn and Johansen, 2013; Wilcoxen et al., 2015). From a scientists' perspective, citizen involvement in observational data collection can facilitate broad reaching studies, which otherwise may not have been possible (Dickinson et al., 2010). A very early example of this was the monitoring of bird migration patterns in Europe initiated by Johannes Leche in the 18th century (Greenwood, 2007). The study required collaboration of amateur scientists in Finland and Belgium to track spring migrants, and this research is still being actively conducted in the region. Similarly, large scale citizen science projects such as the Cornell Laboratory of Ornithology's project NestWatch and E-bird's records of bird sightings are

maintained to approximate population sizes across the United States and world-wide, respectively. Engaging the public in these projects instills a sense of wildlife stewardship (Dickinson et al., 2012). This sense of stewardship will benefit birds and other wildlife as it leads to humans becoming invested in the species' success. This can facilitate the creation of conservation management initiatives geared toward maintaining populations, encouraging responsible resource use, and a sustainable coexistence with wildlife (Shirk et al., 2012). Integrating citizens into my study could be beneficial for simulating recreational bird feeding, especially by establishing nest boxes with feeders in suburban areas. Further it would allow for increased longevity of this study and provide additional insight into this important interaction between humans and wildlife.

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10m

5m

⬤ : Area that was not surveyed

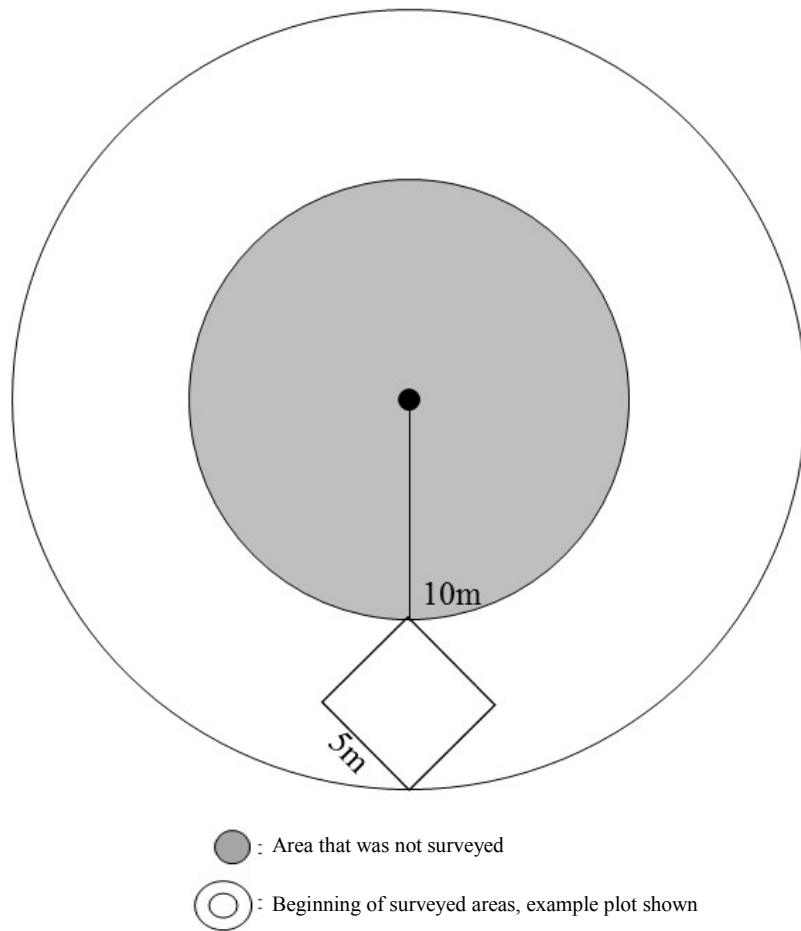◎ : Beginning of surveyed areas, example plot shown

Figure 1. Diagram of invertebrate survey sites around a nest box. The center black dot indicates the active nest box. The gray area indicates areas that were not surveyed in order to minimize disturbance. The white space and beyond indicate surveyable areas. The square within the figure is a representative location of an invertebrate survey plot area.
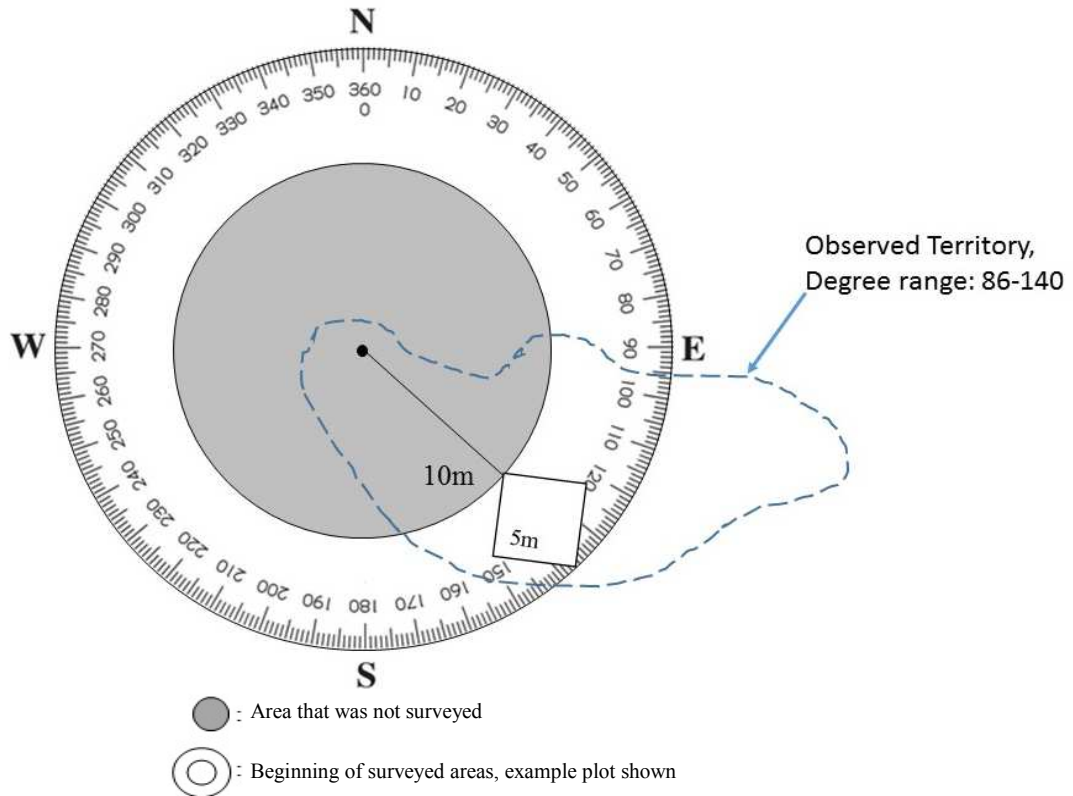
Figure 2. Compass headings were used to determine the location of the invertebrate survey plot area. The implied headings are shown here to illustrate how plot area was randomly selected within a possible territory area. The square within a figure is an example of a plot randomly placed within a degree range on the pair's territory.

Nestbox

(1,1)
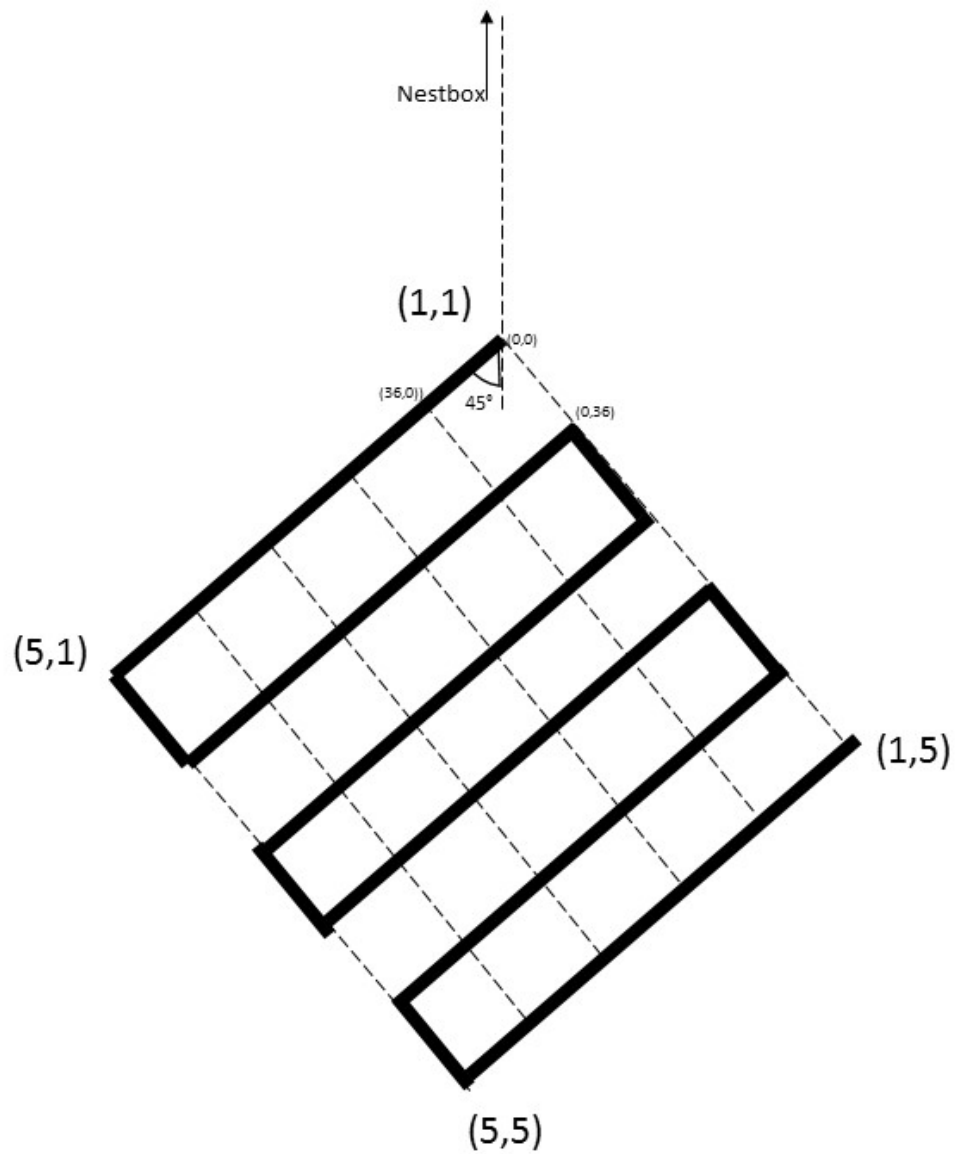
(0,0)

(36,0) 45° (0,36)

(5,1)

(1,5)

(5,5)

Figure 3. Plot area set up for invertebrate surveys. Solid line indicates the physical line (rope) used to set up the grid in the field. Camping stakes were placed at each of the bends in the rope to create the grid. Quadrat labeling within the overall plot area started with (1, 1) at the quadrat touching the 10 m point. Within the quadrat, pitfall locations were points on a traditional coordinate plane with origin being (0, 0).
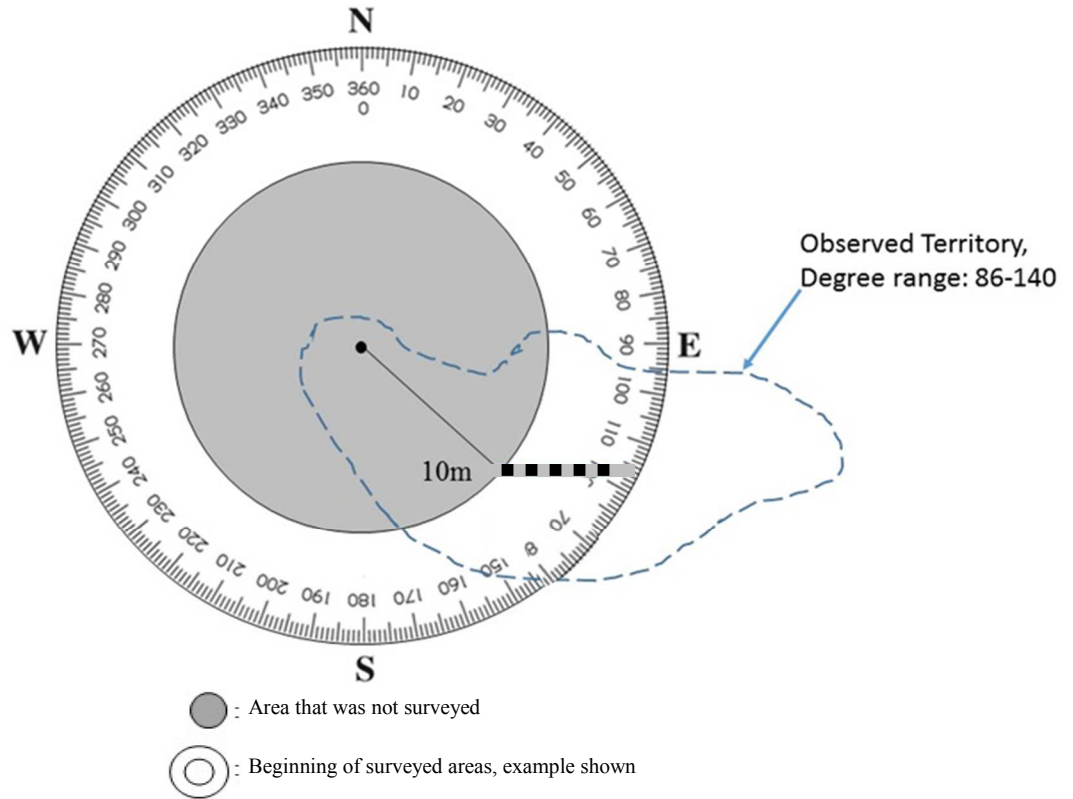
Figure 4. Amended invertebrate survey method for very limited space areas. Black dots on the gray line indicate pitfall traps along the 5 m line.
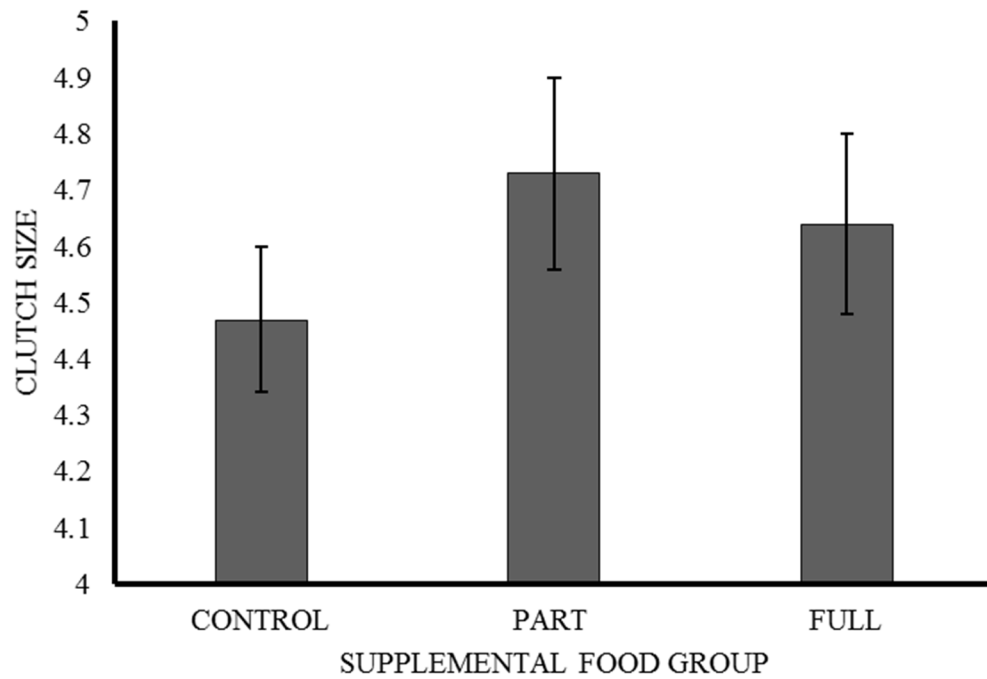
Figure 5. Average clutch sizes in the control, partially supplemented, and fully supplemented groups. Error bars indicate the standard error of the means in each group.

Figure 6. Average hatching success in the control, partially supplemented, and fully supplemented groups. Error bars indicate the standard error of the means in each group.

Figure 7. Average brood sizes in the control, partially supplemented, and fully supplemented groups. Error bars indicate the standard error of the means in each group.
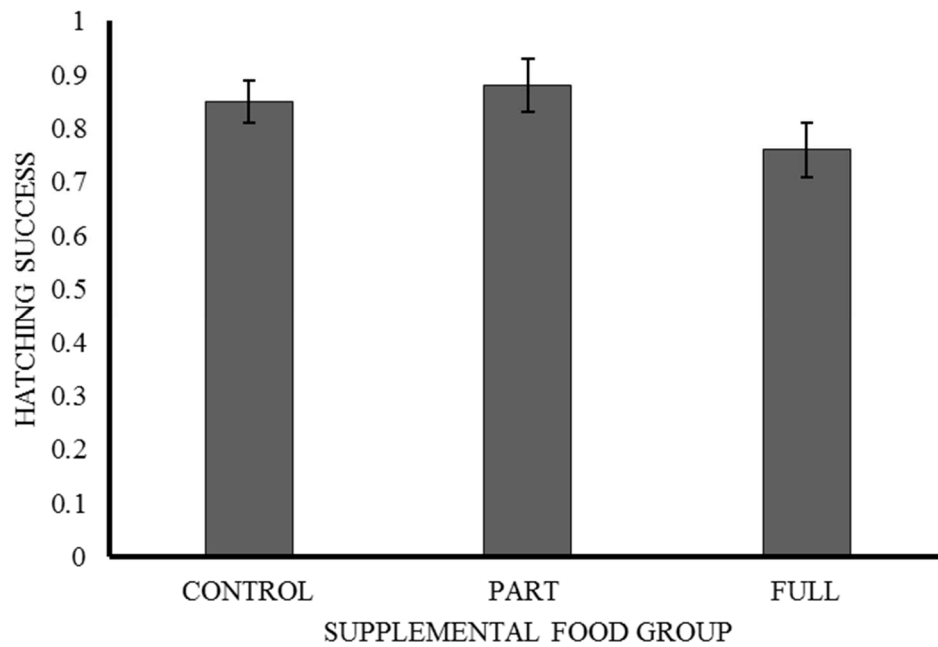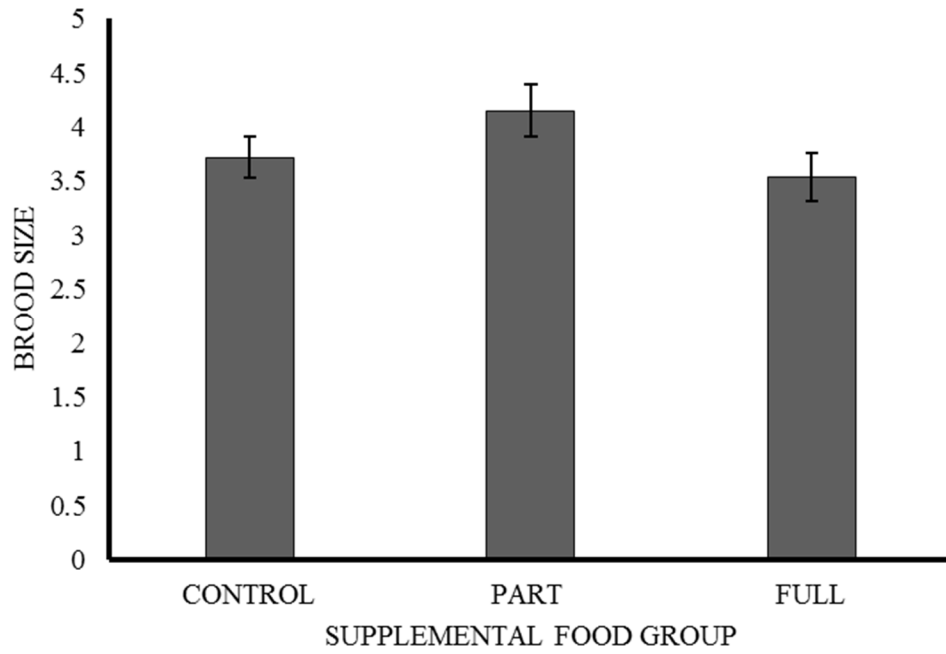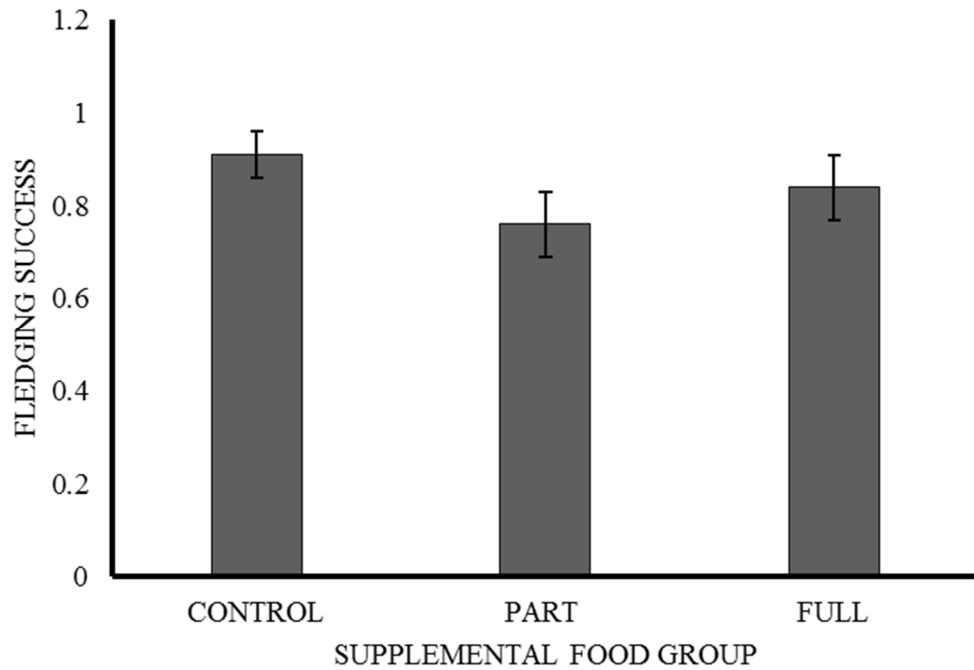

Figure 8. Average fledging success in the control, partially supplemented, and fully supplemented groups. Error bars indicate the standard error of the means in each group.

TABLES

	Diptera	Coleoptera	Orthoptera	Hymenoptera	Hemiptera	Larvae	Araneae
Animal Sciences	10	19	0	0	1	1	1
Agronomy Buildings	0	4	1	1	10	4	13
Botanic Gardens	1	4	1	0	0	0	2
Cross Country	11	33	7	0	12	4	17
Sneed Ranch/ Research Range	0	24	11	4	4	3	8
Highway 51	20	12	30	4	20	7	20
Plant Agronomy	0	1	1	0	0	0	2

Table 1. Abundance of major invertebrate orders observed in both years summarized by trail site. Modeled after (Fay, 2003). Other orders observed: Armadillidae, Blattodea, Cicadellidae, Dictyoptera, Lepidoptera, Phasmatodea, and Zygoptera.

CHAPTER FOUR

CONCLUSIONS

In summary I did not observe any negative side effects associated with supplemental feeding (e.g. Brittingham and Temple, 1986; Fischer et al., 1997; Robb et al., 2008b, Wilcoxen et. al., 2015) or any negative effects associated with food strain previously reported in the literature (e.g., Kitaysky et.al, 2007; Lynn et al., 2003; Lynn et al., 2010). Corticosterone and H:L metrics remained consistent across supplemental groups. Given the multiple modes of activation of corticosterone and its influence on the dispersal of white blood cells, it is likely that supplemental feeding did not significantly affect stress levels of the bluebirds (Davis et al., 2008). Bacterial killing ability was also similar across supplement groups in adults and nestlings. In adults this may have been because of the short period of time between food manipulation and blood sampling, and in nestlings this may have been due to nestlings being largely dependent on innate immune function to combat pathogens (Millet et al. 2007; O'Neal and Ketterson, 2011). Finally reproductive success metrics, specifically clutch size, hatching success, brood size, and fledging success were not significantly influenced by supplemental feeding, nor were they related to my measures of invertebrate order abundance or richness. It would be worthwhile in future studies to

consider land use and weather patterns to determine how these metrics may have also affected food availability. An alternative study design could induce greater food strain, possibly in a captive setting, although this would be less representative of typical bird feeding practices.

Aside from the main objectives of this study, the primary goal of this project was to determine whether providing mealworms as a supplemental food source to Eastern Bluebirds is beneficial. These results suggest that there is not an overall negative effect of supplemental feeding. Many studies reviewed in Robb et al. (2008) found no effect or a positive effect of supplemental feeding. Research gaps existed particularly with regard to physiological changes with food supplementation and how natural food abundance influences supplemental feeding patterns, both of which I aimed to address in this study.

Additional research can be conducted in this area to more fully test for potential negative effects, but it is also important to consider the benefit of engaging the public in nature. With regard to bird feeding, most participants begin with the intention of wanting to help birds (Horn and Johansen, 2013; Wilcoxen et al., 2015). This effect is also seen in recreational spaces, as tourism can attract revenue to a park and benefit the public through education programs on how to responsibly feed wildlife (Newsome and Rodger, 2008). Over the course of this study, I have seen firsthand how easy it is to incorporate citizens into the data collection process and increase public engagement in science. Future studies in this area would benefit from incorporating community

members into the scientific process, and doing so would provide additional, useful data to benefit avian conservation by creating advocates in the community, and community members will learn how they can best protect backyard birds.

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