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Alternative Material Nest Boxes and Impacts on Nestling Physiology and Adult Behavior in the Eastern Bluebird (Sialia sialis)

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Alternative Material Nest Boxes and Impacts on Nestling Physiology and Adult Behavior in the Eastern Bluebird (*Sialia sialis*)

by

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Abstract

In the mid-nineteenth century, Eastern Bluebird (*Sialia sialis*, a native cavity nesting species) populations experienced serious declines because of a decrease in natural cavities and the introduction of non-native, competitive cavity nesting species. The creation of nest box programs led to an increase in bluebird populations and these programs continue to be of importance. In this study, a recycled metal ammunition can was used as a nest box to determine if this was a viable resource for bluebirds. This work investigated the effects of this alternate material nest box on nestling physiology and adult behavior with special emphasis on the impacts of temperature (metal boxes are assumed to be warmer). From mid-February through July 2014, behavioral observations and physiological data were collected at ammunition can and wooden nest boxes being used by bluebirds at Camp Shelby Joint Forces Training Center in Hattiesburg, MS. There were no statistical significance differences between the wooden and ammunition can nest boxes for temperatures, female incubation rates, parental feeding rates, nestling growth measures, or nestling stress levels. However, it was found that offspring who were part of larger broods had higher stress levels and this may be linked to the lower feeding rates larger broods received. These results show that one nest box type was not significantly better than the other, validating the idea that an ammunition can nest box is a safe, alternative habitat for secondary cavity nesters. This may serve as a beneficial resource not only for the species using them but for the work load and finances of property managers deploying nest boxes of this type.

Keywords: Eastern Bluebird, *Sialia sialis*, cavity nesting, alternate materials nest box

Dedication

My parents, Martin and Robyn Jackson:

Thank you for your unconditional love and support through all of my crazy endeavors.

My Honors College adviser, Mrs. Stacey Ready:

Thank you for always believing in me from the very start.

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First, I would like to thank my thesis adviser, Dr. Jodie M. Jawor, for her constant support and guidance through this entire process. Your insight and knowledge of Eastern Bluebirds, how to write a thesis, baking, and just everything in-between has been more than I could ever ask for! Thank you to the U.S. Department of Defense MS Army National Board, for providing the funding for my project, the Camp Shelby Joint Forces Training Center, for allowing me to conduct my research on the base, and Kaylee Gentry and Kim Bagley, for taking part in this project.

I would also like to thank Jacob Garrett, my sisters, Terrie and Kellie Jackson, and my parents for never getting tired of listening to my countless bluebird stories and joining me during box checks and feeding and incubation watches. You all have truly made this experience one I will never forget. This thesis would not have been possible without your love, support, and guidance, and for that I am forever grateful.

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List of Abbreviations

ACNB	ammunition case nest box
WNB	wooden nest box
CORT	corticosterone
ELISA	enzyme-linked immunosorbent assay
USFWS	United States Fish and Wildlife Service
USM	University of Southern Mississippi
DNA	deoxyribonucleic acid
Camp Shelby	Camp Shelby Joint Forces Training Center

Chapter I: Introduction and Literature Review

Approximately 85 species of North American birds use nesting cavities; natural cavities that have resulted from decay, or cavities created by other species, in dead or deteriorating trees (Scott 1977) as a resource for reproducing. Cavity-nesting birds experience intense competition for nesting resources; not only from their own species but from other bird species and even with some mammals. Naturally occurring cavities found in forests are ideally what these types of bird species use, but cavities that are provided through nest boxes are also acceptable (discussed in greater detail below). Finding a suitable nesting cavity is becoming an even more difficult task as habitats that provide natural cavities are being eliminated from forests for a variety of different reasons. Many trees that are ideal habitats for cavity nesters are often removed from forests because they are not esthetically pleasing, conflict with forest management practices, house forest pests, or may be seen as safety hazards (Scott 1977).

As a result of competition and changes in forest management practices, there has been a decline in the amount of available habitats for cavity nesters. Without a cavity, species dependent on this resource cannot reproduce successfully and this can negatively impact population sizes. For example, in the Eastern Unites States, where there has been a decline in forestry practices, Purple Martins (*Progne subis*) depend almost entirely on man-made nest boxes (Soctt 1977) and could suffer severely if this resource is removed. In the past, the Ivory-billed (*Campephilus principalis*) and Red-cockaded (*Picoides borealis*) Woodpeckers have been on the endangered species list, due to habitat destruction (Scott 1977, the Ivory-billed Woodpecker is now considered extinct due to habitat loss). Because of the decreasing number of nest resources, naturally formed or abandoned cavities are highly limited and strong competition between and within species for access to these resources exists, and alternative resources in the form of human produced nest boxes can be an attractive and viable alternative for many cavity nesters (Newton 1994).

The nestling ecology of the cavity nester known as the Eastern Bluebird (*Sialia sialis*) has been well studied. As reviewed by Gowaty and Plissner (1998), the species, more commonly referred to as just 'bluebirds', is recognized by its brilliant blue plumage and its use of nest boxes across the eastern half of the United States, Ontario, and parts of Mexico and Central America. Eastern Bluebirds are open-landscape foragers and prefer a habitat with little or no understory (Gowaty and Plissner 1998). This environment is advantageous as they forage on the ground for small insects and fruits (Gowaty and Plissner 1998).

In the beginning of the mid-nineteenth century, Eastern Bluebirds experienced a decline in population that was thought to have been caused by a reduction in the available natural cavities due to habitat destruction, forest practices that removed snags, and the introduction of other cavity nesters such as House Sparrows (*Passer domesticus*) and European Starlings (*Sturnus vulgarus*) (Radunzel et al. 1997). This decline in the bluebird population led to nest-box program creation and implementation throughout North America in order to increase bluebird reproductive productivity and population size (Radunzel et al. 1997). While these man-made nest-boxes are not exactly like naturally occurring cavities, they provide secondary cavity nesting species with increased availability of nesting sites which ultimately allows for reproduction and potentially an increase in population sizes, as has occurred in the case of the bluebird (Gowaty and Plissner 1998). If nest-boxes are recognized as a particularly valuable resource, then they could potentially cause competition within populations. Here we focus on Eastern Bluebirds using human-provided nest boxes and investigate whether the type of nest box offered impacts behavior and physiology in the individuals using them.

Evidence that proves nest site limitation can cause an increase in competition was found in a field study using another secondary cavity nester, the Blue Tit (*Cyanistes caeruleus*) (Jacot 2009). Researchers experimentally limited nest site availability in some plots (experimental plots) and then compared these to control (unmanipulated) plots. They found that the limitation of nesting sites increased competition between individuals allowing high-quality, more aggressive, males to out-compete lower quality, less aggressive male birds for nest box access (Jacot 2009).

The results of Jacot (1009) show not only that the decreased number of nest boxes led to competitive interactions between males but also suggest that nest site availability could have impacted a female bird's view of a male bird's quality. It was thought that the competitive interactions among males and the fact that certain males out-competed others caused the females to perceive the successful males, the ones who ultimately obtained the nesting resources, as higher quality mates (Jacot 2009). Nest site limitation did have an effect on reproduction. It was shown that in the experimental plot females invested more in offspring feeding than control plot females. This could be a result of larger territories having a greater abundance of food, but it could also be because females may strategically feed more when breeding with a male that is seen as high quality (Jacot 2009). While there was an increase in female feeding rates, the increase was not significant, which could lead to the assumption that males may have even reduced their investment in offspring feeding as opposed to females increasing their feeding rates independently (Jacot 2009). This, and other, studies show that the use or limitation of nest-boxes can lead to more than just competitive interactions for access to the nest boxes. They can potentially cause changes in the amount of time and energy that individuals invest when raising their offspring.

Impacts of increased competition for nest boxes was also seen in a study conducted by Meek and Robertson (1994) that analyzed how interspecific and intraspecific competition for nest boxes affected mate guarding in the Eastern Bluebird. It was found that nest site competition between Eastern Bluebirds and Tree Swallows (*Tachycineta bicolor*) was most intense on multi-box territories rather than single-box territories, swallows intruded more on multi-box territories. It was also found that non-resident male bluebirds intruded on both multi-box and single-box territories, and at both territories (multi-box and single-box) the resident male bluebirds spent significantly more time guarding their mates, especially when their mates were fertile (this helps ensure paternity of offspring).. Even though it was found that the male bluebirds spent significantly more time guarding their mates, when comparing activity between multi-box and single-box territories, the males spent significantly less time guarding their mates at multi-box territories. This could be due to males on multi-box territories also having to defend against Tree Swallow invasion.

In a study conducted by Duckworth (2006), the use of intraspecific aggressive behavior when competing for nest–boxes was analyzed specifically in Eastern Bluebirds. Nest boxes were placed at different densities in experimental plots and competition for these boxes was observed. Increased competition for areas with a high density of nest boxes caused the birds to be separated into different breeding habitats based on the selection for different behavioral traits. Specifically, it was found that male bluebirds demonstrating higher levels of aggression obtained territories with more nest boxes on them.

A separate study was conducted by Brawn (1984) that focused on the defense of nest boxes by Western Bluebirds (*Sialia mexicana*) during the post-breeding period. Nest boxes were installed on three study plots about 40-60 km apart just south of Flagstaff, Arizona and during four breeding seasons, the nest boxes were checked to see if they contained nests. Similar to other studies involving nest boxes, in this study, it was found that intraspecific competition may have been taking place. The data shows that birds preferred nest boxes over natural cavities, and breeding densities increased in the years that the nest boxes had been installed on the plots. In order to further determine whether intraspecific competition is taking place here, installing more nest boxes on the plots would be a reasonable method to test this hypothesis.

Taken together the studies outlined above show that nest boxes are viable, and even desirable, resources for nesting among cavity nesters and the placement of these boxes can impact behavior in the individuals using them. But, the amount of nest boxes in a specific area is not the only factor that can contribute to making nest boxes a valuable resource. Factors like the design, material, and quality of a nest box play a significant role in determining how valuable a resource the nest box can be and whether it can impact adult behavior (Swaddle et al. 2012).

Stanback and Rockwell (2003) investigated the hypothesis that an Eastern Bluebird's decision to switch nest sites depends not only on the current state of the nest box they are inhabiting, but also on the quality of alternative nest cavities currently available to them . The study was conducted by providing bluebirds with alternative cavities that were of higher and lower quality than the nest-box that they currently inhabited. These alternative cavities included different combinations of soiled, clean, preferred material, and non-preferred material nest boxes. The nest boxes were determined to be preferred and non-preferred from a choice experiment where the bluebirds were provided with attractive and unattractive nest boxes. More specifically, the birds were given the option of a woodcrete nest box or a wooden nest box; the woodcrete was the more preferred type (Stanback and Rockwell 2003). During one of the experiments, the bluebirds were forced to choose between their soiled box of the preferred type (woodcrete) and a clean box of the less preferred type (wooden). It was found that a significant amount of bluebird pairs chose to reuse their original, preferred material nesting cavity, even though it was not in the best condition (contained soiled nest material). In another experiment, the bluebirds were given the choice between their soiled box of the preferred type (woodcrete) and a clean box of the exact same type. Here, it was found that a significant number of bluebirds switched to the clean box that was still of the preferred type.

These results showed that the bluebirds do not just switch nest boxes when the quality of their own nest box is reduced and becomes soiled with used nest material. The quality of the alternative nest box is also taken into consideration and weighed against the quality of the nest box that the bird currently resides in. This study shows that bluebirds are willing to switch their nest sites if they recognize an alternative nest site to be more valuable than their own and that they could recognize alternative materials besides wood (in this research it will be a recycled aluminum ammunition can) as preferred. For this reason, it can be assumed that if bluebirds recognize our alternative material nest boxes as a valuable resource, then they will be willing to leave their previous nest site or work to defend it at a higher level.

My research will be focusing on analyzing the effects of traditional wooden nest boxes versus non-traditional metal nest boxes on adult behavior and nestling physiology in the Eastern Bluebird. In our study the non-traditional metal nest boxes will be constructed from empty ammunition cases, and we predict that the non-traditional metal nest boxes will prove to be a resource that the bluebird population at Camp Shelby Joint Forces Training Center (location of research) will recognize as valuable. Previous work with these boxes has suggested that they do not get any warmer than traditional wooden boxes, and do not seem to fluctuate strongly in temperatures throughout the day and night (C. Potin, pers. comm.). This lack of fluctuation may be beneficial to the adults and nestlings using these boxes. It has the potential to lead to faster growth rates in the nestlings which could result in the need for less parental investment in terms of brooding and more parental investment in terms of nestling feeding.

The impact of temperature is of particular interest here. In a recent study by Lynn and Kern (2013), Eastern Bluebird nestlings were subject to environmentally relevant periods of cooling to determine if corticosterone (CORT), a common stress hormone, was secreted due to decreases in temperature. Elevated CORT levels in nestlings can slow body growth and impact neurological development (reviewed in Groothius and von Engelhardt 2005). Four different treatments that simulated different environmental temperatures were used in the experiment. It was determined that the cooling bouts the chicks experienced caused an increase in their CORT levels. This experiment shows how changes in temperature can have a negative effect on the stress levels of bluebird nestlings (Lynn and Kern 2013). Although a decrease in temperature is something that bluebird nestlings experience in their natural environment due to brooding females being away from the nest, exposure to stressful stimuli early in development can contribute to phenotypic variation that will affect the bluebird nestlings as they continue to grow. Because alternative-material nest boxes and wooden nest boxes have previously noted differences in temperature consistency, stress levels in nestlings are expected to vary due to these differences.

In my work, by analyzing and comparing the nestlings' stress hormone levels and their physiology (here body growth), I will be able to determine whether or not the alternative material nest boxes are a suitable, alternative form of housing for Eastern Bluebirds. This, coupled with potential changes in adult behavior (potentially leading to the perception that the boxes differ in value) for those using the alternative material nest-boxes, comprises the focus of this work.

Chapter II: Methodology

The metal ammunition case nest boxes (hereafter ACNB) were produced by the Environmental Office at Camp Shelby Joint Forces Training Center (The Nature Conservancy, Camp Shelby Conservation Program, CSJFTC-ENV Building 6530 Camp Shelby, MS 39407-5500, (601) 558-2875) and placed along with traditional wooden nest boxes (hereafter WNB) in different locations off of Lee Avenue running east from the North Gate and around Dogwood Lake of Camp Shelby Joint Forces Training Center (hereafter Camp Shelby) during mid-February 2014 (see Fig. 1 showing nest box types). The initial nesting activity that took place at each of these nest boxes was observed through scheduled watches and box checks that took place twice a week through the end of February to the end of March 2014. Activity at both the WNBs and ACNBs was observed and compared throughout the breeding season. In order to individually identify birds, we caught adult bluebirds (either during nest box acquisition, incubation, or nestling feeding) and placed U.S. Fish and Wildlife Service (USFWS) serial numbered bands and colored plastic bands on them. During this time, we also collected from adults' standard morphometric data and blood for future hormone or DNA analyses.

During the course of the project, nests were stage matched, and the stage matching was initially to be based on egg laying dates of pairs of birds using the two types of nest boxes, so that the data would be more easily comparable (see Results for modifications). If there were pairs using both types of nest boxes through the season (e.g., they initiated a nest in a WNB and then switched to an ACNB later, or the reverse), these pairs were monitored for the number of eggs they produced in the clutch and the initiation of the incubation by the female for comparison of individuals using both box types.

Figure 1. A traditional wooden nest box (on left) and a metal nest box (right) made from a painted, recycled ammunition case

As the incubation period began, pairs in both types of nest boxes were monitored for incubation rates, and this focused solely on the female bluebirds since males do not participate in this activity. Methods used to determine incubation rates were based on the technique used previously (Jawor and Breitwisch 2006) when working with Northern Cardinals (*Cardinalis cardinalis)*. Incubation rates were based on two (2), one hour observations done on consecutive days. The observers sat approximately 30 meters from the nest and observed when females left or returned to the nest box, how long they were gone from the nest, and how long they were in the nest box.

After the incubation period, when the eggs hatched, parental care behavior and nestling information was gathered. During this part of the experiment, we focused on the number of eggs that hatched in each clutch and the length of time that it took the eggs to hatch (the number of days between laying and hatching, while taking incubation behavior into account). Number of days until hatching will not be reported on further as there were no differences between clutches in box type for this parameter. Parental feeding rates were monitored in pairs using ACNBs and WNBs. Since both male and female bluebirds participated in this activity, the feeding rates for both males and females were measured. This started on day three (3) of nestling life. Three (3), one (1) hour watches were done on consecutive, and feeding rates were calculated after these watches. Similarly to incubation observations, observers sat approximately 30 meters from the nest and observed when pair members came to the nest with food. The methods used to determine feeding rates were based on the methods used by Jawor et al. (2003) in work with cardinals. Feeding rates were based on the number of parent trips and the number of offspring in the nest (larger broods require more parental trips); feeding rates were analyzed as feeds/nestling/hour. We did not correct for nestling age (older nestlings are typically fed more, Jawor et al. 2003) because all observations were conducted on days 3-5 of nestling life for all broods.

As the nestlings began to grow, the growth rates of the nestlings in ACNBs and WNBs were compared. This was done by measuring mass and tarsus length of nestlings on day three (3), (at the beginning of parental feeding rate observations) on day six (6), and on day twelve (12) of nestling life. Growth rates were compared to brood size and parental feeding rates. To facilitate individual recognition of nestlings, nestlings were marked with non-toxic paint on the leg, bill, and back. On day twelve (12), nestlings received a USFW serial numbered band and had a small, non-lethal blood sample collected for the analysis of the stress hormone, corticosterone (CORT).

In order to compare temperatures experienced to behavior and physiology, the temperature in all of the nest boxes was tracked using a temperature recording iButton (Embedded Data Systems, LLC, Lawrenceburg, Kentucky, USA). This temperature information was compared to the behavioral data that was collected from the birds. Data loggers were placed into nests during incubation, following incubation behavior data collection, and remained in the nest for up to five (5) days.

Near the end of the experiment, fledging dates for offspring from ACNBs and WNBs were compared to determine if offspring fledge faster or slower in one type of nest box; we will not report on this further as there were no differences in fledgling dates. Re-nesting behavior of the adults was assessed through observation of pair behavior and egg-laying at the nest boxes included in this study (both the nest box they just used or boxes in the immediate vicinity). Immediately upon fledging, nest materials were cleaned out of boxes so that if pairs re-nested they would potentially use the same nest box. If the pairs did not use the same box, attempts were made to determine where pairs were on Camp Shelby grounds.

Once the field data was collected, the lab analyses were completed. These include ELISA immunoassays for CORT (Arbor Assays, Ann Arbor, Michigan, USA), behavioral data from adults, growth data from nestlings, and statistical analyses. The methods that were used to do the immunoassays were based on the methods used previously (DeVries and Jawor 2013) and involve extraction of hormones from plasma samples (three extractions using diethyl ether) and the analysis of extracted samples in duplicate sub-samples following kit directions.

The CORT ELISA uses an antibody coated microliter plate that captures CORT from extracted samples. This is a 'sandwich' type assay where additional antibodies containing color changing domains are incubated with hormones from extracted samples. A complex of CORT molecules from samples between two antibodies (one attached to the plate for capture and a second with color changing ability) is formed and variation in the intensity of color change in the microtiter wells gives an estimation of CORT in the original extracted sample.

All findings were analyzed using SPSS 20 (IBM) and analyses consisted of T-tests to compare variables between ACNBs and WNBs and non-parametric statistics when comparing individual components (e.g., tarsus length and circulating CORT in nestlings). All work was approved by the USFWS Bird Banding Laboratory (permit # 23479), Mississippi State Department of Wildlife, Fisheries, and Science (permit # 0603142) and USM's Institutional Animal Care and Use Committee (protocol # 12042601).

Chapter III: Results

At the end of the breeding season, it was determined that nests would be stage matched based on behavioral sequence rather than by date, due to low nest occupancy throughout the study site. Additionally, it was determined that there would be no comparison between pairs using both types of nest boxes since no pairs switched between box types and the only re-nesting pair used the same box.

Box Occupation and Temperature

In the five (5) WNBs and four (4) ACNBs that were inhabited, the average, minimum, and maximum temperatures for the boxes were recorded using a temperature recording iButton. The data from the iButton showed that there were no significant differences in the temperatures based on box type. The minimum temperatures compared between ACNBs and WNBs showed that there was no significant difference between box types (Independent sample T-test: $t = 0.0001$, $df = 10$, $P = 1.00$). The same was found when maximum temperatures were compared between box types ($t = 0.04$, $df = 10$, $P = 0.27$). However, the data showed that the maximum temperature in the ACNBs tended to be higher than the maximum temperature in the WNBs, but not significantly ($t = 0.04$, $df = 10$, $P = 0.96$, See Fig. 2).

Box Type and Parental Care Behavior

Incubation Behavior: When incubation behavior was compared between box types, it was found that females incubated at the same rate regardless of box type ($t = 0.28$, $df = 7$, P = 0.78). In the next two analyses, since there were no differences between box types for incubation or for temperature data were not separated based on box type. The

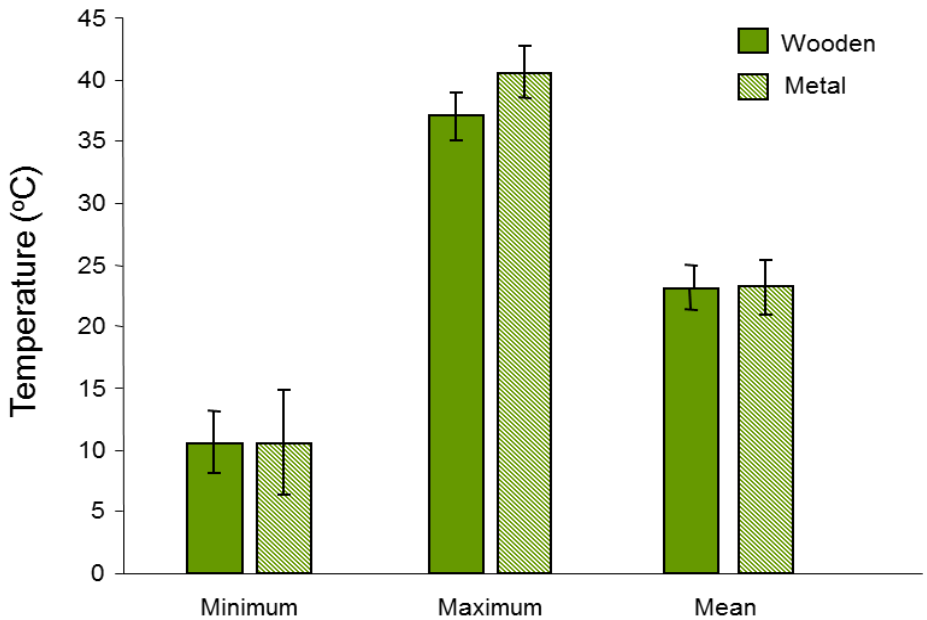

Figure 2. Temperatures in metal and wooden nest boxes over the course of early incubation.

incubation behavior of the bluebirds was compared to the minimum temperatures of all of the boxes, and it was found that when temperatures are lower, females tend to incubate more (Spearman rank correlation: r_s = -0.74, p = 0.02, n = 9). Incubation behavior was also compared to maximum temperature, and there was no effect of high temperatures on incubation ($p > 0.05$). Incubation behavior was compared to the average temperature, and it was found that when average temperatures are lower, female bluebirds tend to incubate more (r_s = -0.81, p = 0.008, n = 9, See Fig. 3).

Feeding Behavior: When the number of nestlings produced in each brood was compared to box type, no significant differences were found based on whether birds nested in ACNBs or WNBs (t=0.22, df = 9, P = 0.82, See Fig. 4). The feeding rates between members in a pair were compared, and the data showed that mated pairs fed their nestlings at a similar rate (r_s = 0.93, $p < 0.0001$, n = 9). The number of nestlings in each box type was compared to the feeding rates of males and females. It was found that the number of nestlings did not affect parental feeding behaviors (females: r_s = -0.47, p = 0.20, n = 9; males: r_s = -0.48, p = 0.18, n = 9). Lastly, male and female feeding rates were compared between box types, and it was found that there was no impact of box type on male (t = 1.63, df = 7, P = 0.14) or female behavior (t = 1.66, df = 7, P = 0.14, See Fig. 4). The higher feeding rates observed in metal boxes in Fig. 4 can be linked to one pair that fed their nestlings in excess of 10 times an hour (5+ feedings/parent).

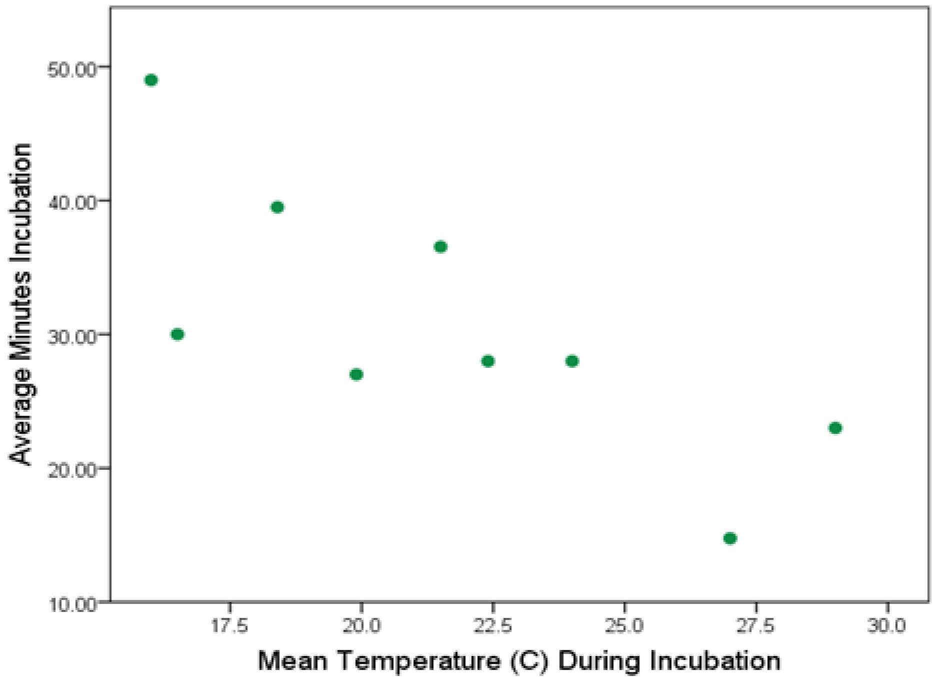

Figure 3. Average minutes females incubated during observations and mean temperature (°C) during incubation period.

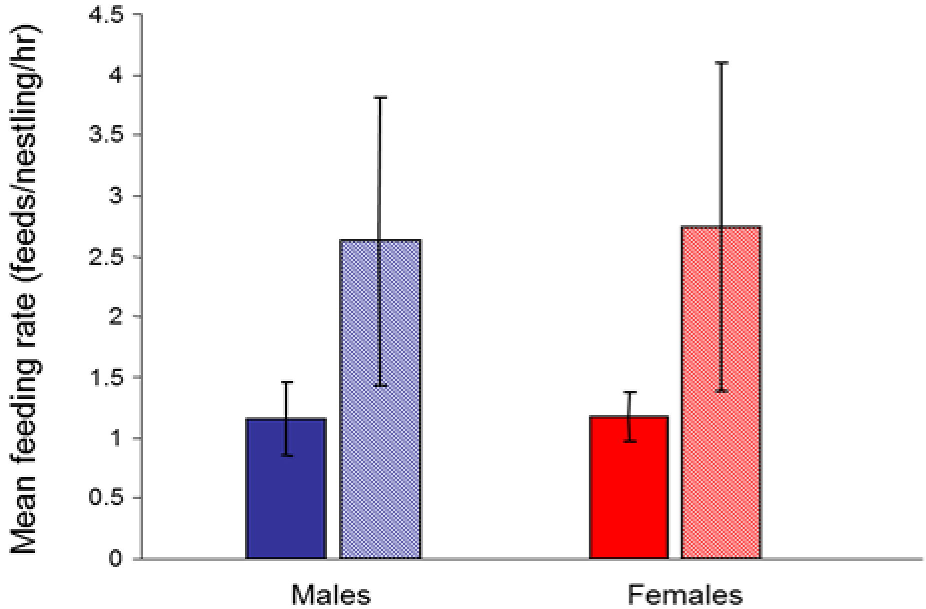

Figure 4. Feeding rates in male (blue) and female (red) bluebirds. Data for wooden boxes in solid colors and data for metal boxes is hatch ed.

Nestling Physiology, Box Type, and Parental Behavior

A comparison between the nestlings' tarsus growth rate and box type (t=0.52, df = 4, P = 0.62) and mass increase rate and box type (t = 0.55, df = 4, P = 0.60) showed no influence of box type on nestling growth rate parameters. Although the type of nest box did not affect the tarsus growth rate or mass increase rate, when last day size measures of the tarsus (e.g., day 12 of nestling life) and mass were compared to the box type, nestlings in wooden boxes tended to have longer tarsi (t = 1.88, df = 30, P = 0.06). The effects of feeding behavior on tarsus growth rate and mass increase rate were also analyzed. Male feeding behavior showed no impact on tarsus growth rate (r_s = -0.10, p = 0.87, n = 5) and change in mass (r_s = 0.40, p = 0.50, n = 5). Similarly, female feeding behavior also showed no impacts on tarsus growth rate (r_s = -0.70, p = 0.18, n = 5) and mass increase rate (r_s < 0.0001, p = 1.0, n = 5) in nestlings.

Corticosterone levels in the nestlings were compared to growth rate as well, and it was found that their CORT levels did not have an impact on tarsus growth rate (r_s = 0.42, p = 0.39, n = 6) or the mass increase rate (r_s = 0.60, p = 0.20, n = 6). To further investigate the growth rate in nestlings, a comparison between the nestling number and growth rate for tarsus (r_s = 0.52, p = 0.28, n = 6) and change in mass (r_s = 0.18, p = 0.72, n = 6) was conducted; it was found that the number of nestlings in the nest had no effect on the growth rate.

Nestling Corticosterone, Box Type, and Parental Behavior

Box Type: When the corticosterone levels between broods in ACNBs and WNBs were compared, it was found that there was no significant difference in brood CORT levels based on box type (t = 0.26, df = 9, P = 0.79). Brood size was also compared with brood CORT level, and the data showed that larger broods tend to have higher average levels of CORT (r_s = 0.71, p = 0.015, n = 11, See Fig. 5).

Nestling impacts: Nestling body size measures that were taken shortly before fledging were compared to CORT levels, and the data showed that there was a relationship between tarsus length and corticosterone level, with nestlings with longer tarsi having higher corticosterone levels (r_s = 0.35, p = 0.05, n = 32, See Fig. 6). There was no correlation between the nestlings' CORT levels and mass (r_s = -0.08, p = 0.62, n = 32). Lastly, brood CORT levels were compared with both male (r_s = -0.64, p = 0.05, n = 9, See Fig. 7) and female (r_s = -0.68, p = 0.04, n = 9, See Fig. 8) feeding rates. In both sexes, the data showed that when adult birds fed their broods less, the broods had significantly higher levels of CORT.

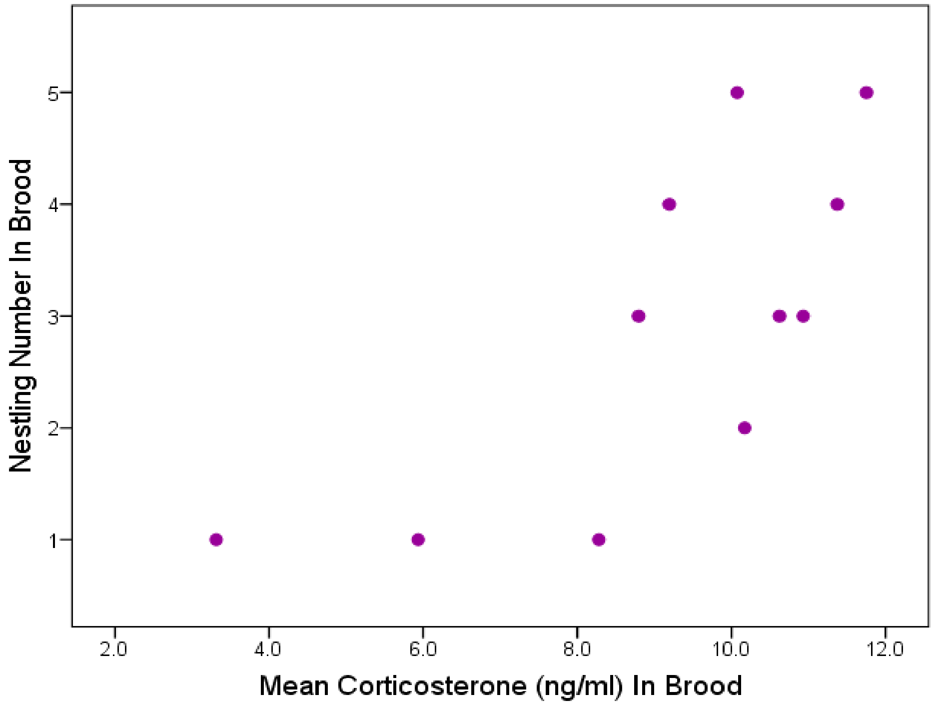

Figure 5. Mean corticosterone level in broods compared to the number of nestlings in the brood.

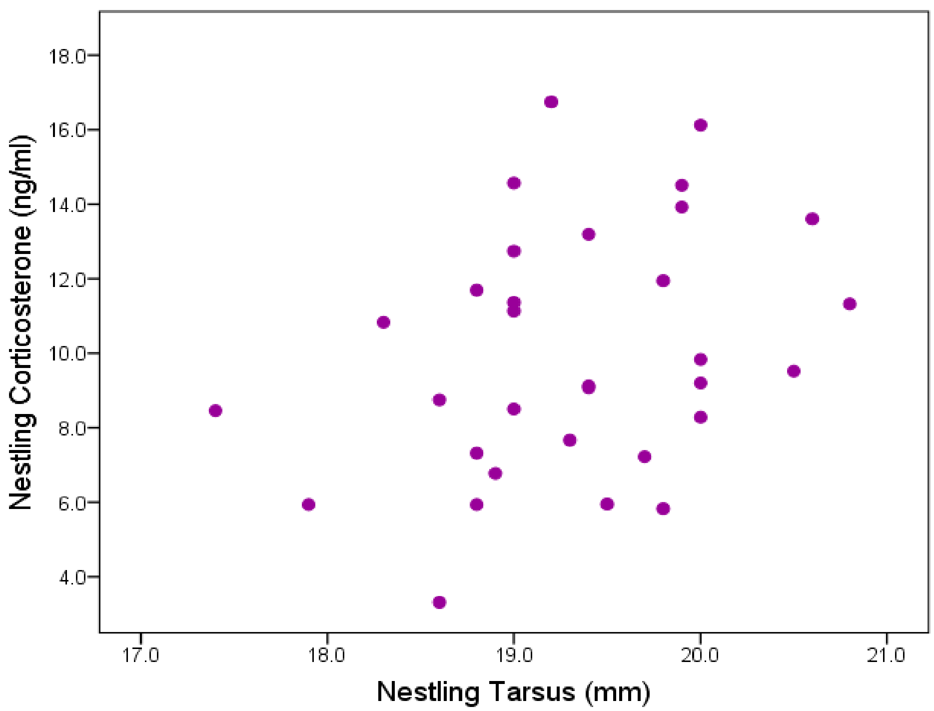

Figure 6. Nestling body size (tarsus) and individual levels of corticosterone (values collected at day 12 of nestling life).

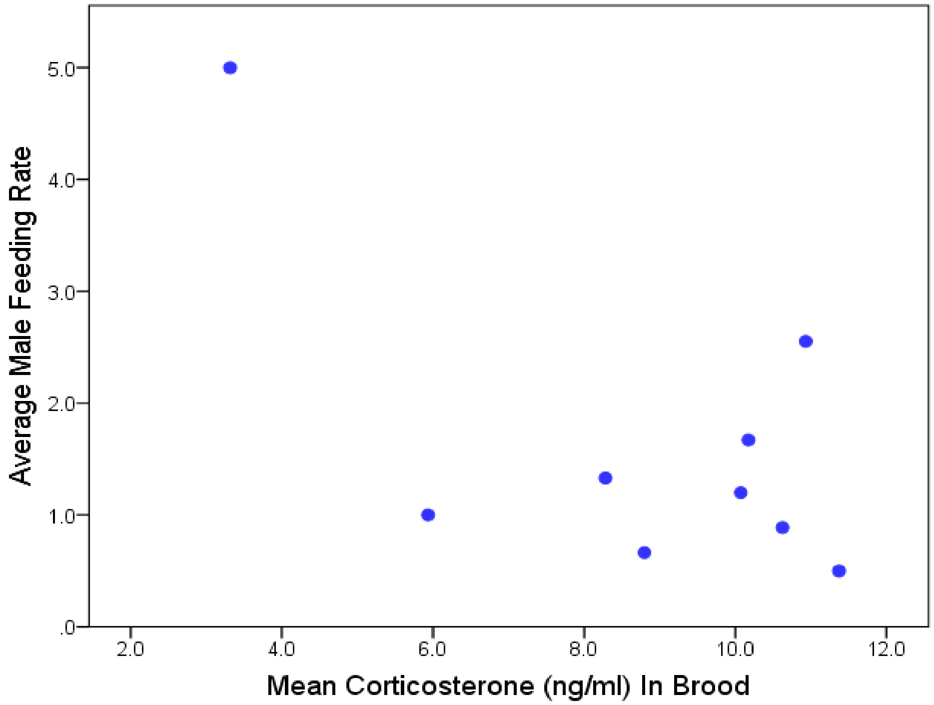

Figure 7. Male feeding rate (feeds/nestling/hour) and the mean corticosterone level for nestlings in same brood.

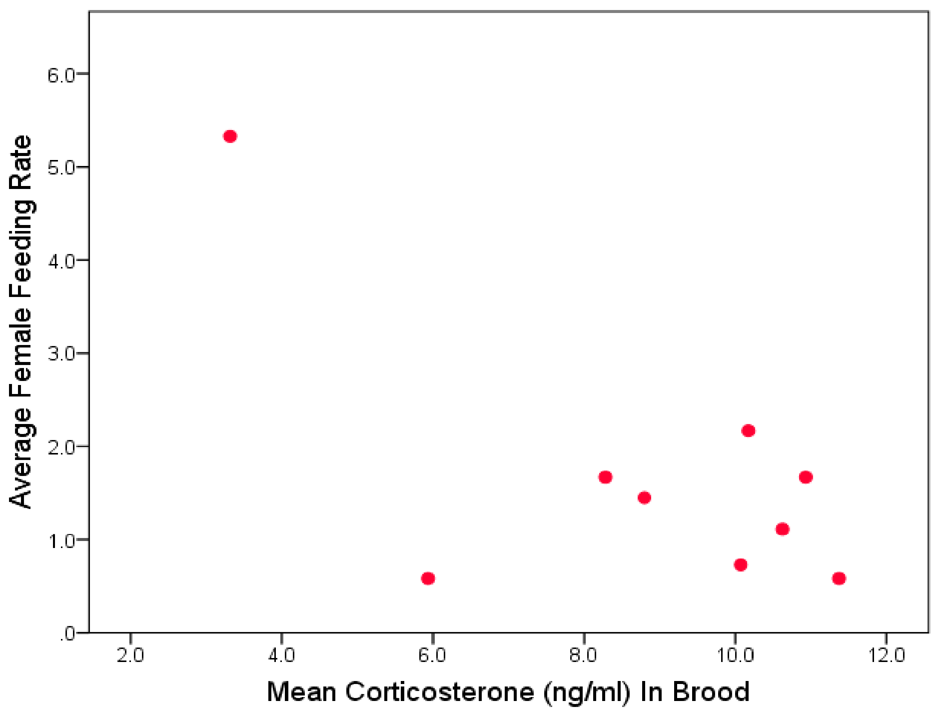

Figure 8. Female feeding rate (feeds/nestling/hour) and the mean corticosterone level for nestlings in same brood.

Chapter IV Discussion:

Because there were two different nest box materials used in this study, it was predicted that there would be a temperature difference between the boxes. It was found that the temperatures did not differ according to the box type, and in each type temperatures fluctuated equally throughout the day, contrary to what was previously found (see Fig. 9). We found that the amount of times the female bluebirds incubated was based on the temperature of the box, rather than the box material. Female behaviors in ACNB did not differ from WNB, but when temperatures were lower females incubated more in both box types. The parental care of the bluebirds was not affected by box type, and both male and female bluebirds within pairs fed their nestlings equally. This suggests the birds did not see the metal or wooden nest boxes and the broods within as differing in value. Along with the box type, it was found that the parental feeding rate was not impacted by the number of offspring; pairs did not adjust their provisioning effort when faced with more young to feed. Offspring that were part of larger broods were more stressed (higher CORT), but there was no effect of box type on the brood size or stress levels of the nestlings. However, it was found that the broods of parents who provided less care had higher stress levels.

Although our data showed that the ACNBs were not significantly different from the WNBs, there are many other factors that need to be investigated in order to fully determine if ACNBs could be seen as higher quality. In many past experiments dealing with varied nest cavity situations, the competition that took place between the birds for nest boxes was analyzed. For example, in the study conducted by Jacot (2009), the decrease in the amount of nesting sites caused an increase in competition, ultimately

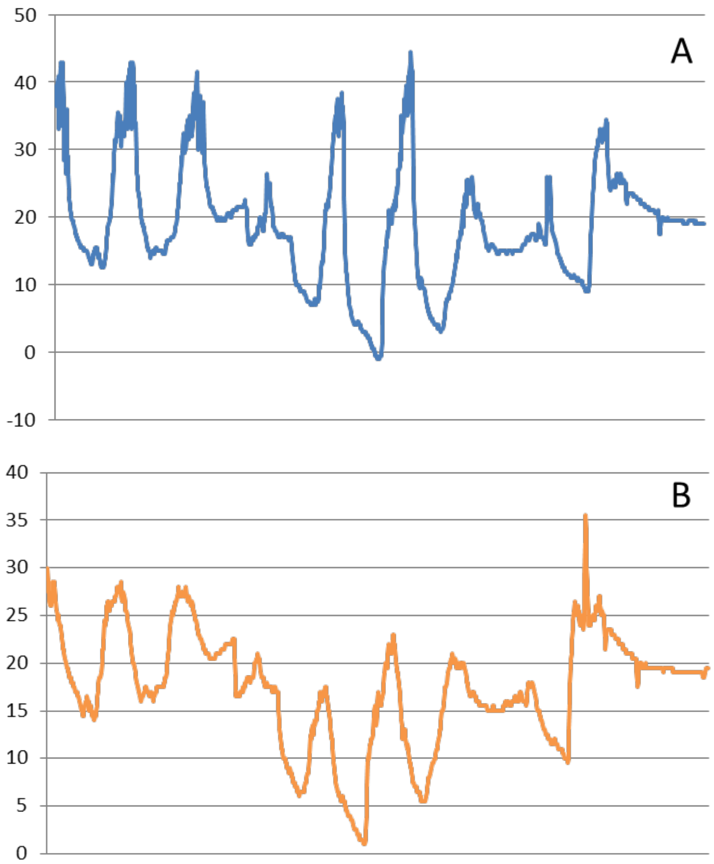

Figure 9. Temperatures over one week in a metal box (panel A, top) and in a wooden box (panel B, bottom).

allowing high-quality male birds to outcompete lower quality male birds (Jacot 2009). Perhaps if the number of ACNBs on the plot that was used in our experiment would have been limited, the ACNBs would have appeared more attractive to the bluebirds. However, there have been other studies that have shown that plots with an increased amount of nest boxes have the potential to cause increased competition as well. In the study conducted by Meek and Robertson (1994), it was found that competition between Eastern Bluebirds and Tree Swallows was most intense on multi-box territories rather than single-box territories. This study shows that birds who do nest on territories with more than one box located in the general vicinity could possibly see these territories as more beneficial, causing them to compete for these areas before they compete for territories with areas that have a single box. Because of this study, it can be presumed that an increase in the amount of nest boxes on certain territories could have caused an increase in competition within our experiment. In the future, increasing or limiting the number of nest boxes in certain areas of the experimental plot could have the potential to cause a noticeable increase in competition, ultimately allowing for us to observe and determine if one nest box type is favored over the other.

In a study by Kozlowski and Ricklefs (2011), the effects of brood size on growth and stress levels in Eastern Bluebird nestlings was analyzed. The relationship between brood size, growth, and steroid hormone production in Eastern Bluebirds was analyzed by cross-fostering nestlings into small, medium, and large broods. Nestling characteristics such as body mass, skeletal size, serum testosterone, and baseline serum corticosterone concentration were measured prior to fledging. Nestlings that were raised in larger broods weighed less than nestlings who were raised in the small and medium-sized broods, and they also secreted elevated concentrations of testosterone (Kozlowski and Ricklefs 2011). While nestling corticosterone concentrations did not change in response to brood size or body condition, concentrations were higher in adult males rather than females (Kozlowski and Ricklefs 2011). The data of the study showed that nestlings experiencing nutritional and social stress tend to increase testosterone production (Kozlowski and Ricklefs 2011). Because the nestlings of larger broods secreted elevated levels of testosterone, it was assumed that they were experiencing nutritional and social stress as well although this was not reflected in a hormone of stress.

Similarly to Kozlowski and Ricklefs (2011), our research also shows that nestlings who are part of larger broods potentially experience competition for parental care resources. Naturally, nestlings who are a part of larger broods are going to experience reduced parental care because there are other nestlings in the brood that need care as well. This reduction in care is most likely seen as lack of food, since the parents in our study did not adjust their parental care according to the number of offspring. In larger broods, who were receiving less food, we saw higher levels of CORT, with does not agree with the findings of Kozlowski and Ricklefs (2011). Potentially the age of nestlings when blood samples were collected (we collected blood at an earlier age) is a factor, or larger issues such as the quality of the food being delivered to nestlings is acting as a factor. However, there could be other aspects of behavior, physiology, or environment that played a role in the increased stress levels observed here.

A second study by Davis and Guinan (2014) analyzed the relationship between parental behavior and corticosterone levels of mates and their nestlings. It was found that the number of overall feeding trips that were made by both the male and female parents were positively correlated to the corticosterone levels of nestlings (Davis and Guinan 2014). It can be assumed that the number of feeding trips that both the male and female parents made resulted in the stress level of the nestlings. This idea that parental care has a direct effect on the stress levels of nestlings can be reaffirmed through our experiment which showed when nestlings receive less parental care, they generally had higher stress levels. However we had a reverse association (more trips led to less CORT) when compared to Davis and Guinan (2014) and the quality or quantity of food delivered (something we did not assess and which could vary between study sites) could be a factor. Potentially in Davis and Guinan (2014) elevated CORT is facilitating growth (something we initially predicted for our study) and a compounding factor such as food quality led to the findings we see in our study. The increased stress levels of our nestlings is alarming in the fact that experiencing this stress during the developmental time period can cause unwanted side effects later on in the bluebird's life.

In conjunction with the short-term stress response that is induced during a stressful situation, pathological effects of chronic stress can occur if the stress extends for longer periods of time. Pathological effects caused by chronic stress can involve and affect cardiovascular, metabolic, reproductive, digestive, immune, anabolic, and psychological processes (Nelson 2005). It has been hypothesized that stressful situations early in life can affect reactions to stress later in life. However, not just any type of stressful situation can have an effect on an adult bluebird. The severity and amount of time that stress is induced determines whether or not an effect will be observed in adulthood (Nelson 2005). For example, it has been found that post-natal exposure to mild stressors blunts adult stress responsiveness. This altering in stress responsiveness early on in life could affect the behavior of adult bluebirds and potentially their survival. In future studies, continuing to observe whether or not the increased stressed levels of the bluebird nestlings observed here has an effect on bluebird behavior later on in life and whether or not these stress levels have anything to do with box type would enhance our understanding of whether the metal boxes had any sort of impact on the individuals raised in them.

Along with observing the progression of nestling stress levels for those who reside in both ACNBs and WNBs, differences in future settlement could also be observed. In future breeding seasons, looking at settlement behavior in comparison to the previous breeding season would allow us to see if the bluebirds preferred one nest box type over the other. Increased competition and the possibility of birds switching from WNBs to ACNBs between seasons, would help us to confirm whether or not the ACNBs could be viewed as higher quality. Observing whether pairs who inhabited the ACNBs return to this type of nest box during the following breeding season would also help to determine if there may be a significant difference between ACNBs and WNBs. Bluebirds will return to the same general nesting population after a non-breeding season break (Gowaty and Plissner 1998) and potentially those who used ACNBs may be more faithful to this box type and location where this resource is found, this could have longer term impacts on population genetic structure and adult behavior.

Even though no significant differences between the ACNBs and WNBs were found for the standpoint of individuals using the boxes, the ACNBs are potentially more beneficial from a manager's standpoint. Throughout the field work of our experiment, it was observed that the metal boxes did not show any type of rusting or decaying. Because the metal boxes are likely to last longer than the wooden boxes that could potentially rot or become deteriorated due to weather conditions, the metal boxes are more beneficial financially. In addition to being longer-lasting the metal boxes are cost effective considering they are recycled ammunition cans from the military base (a readily produced quantity) and may otherwise not be put to use, this is a resource that has already been paid for and is not something that property managers on military bases would need to purchase again. Additionally, this resource could be sold to non-military operations that are in need of a more durable nest box design. While both ACNBs and WNBs were both inhabited wasps (*Polistinae* spp.) at some point during the breeding reasons, wasps tended to be more attracted to the wooden boxes rather than the metal boxes, which is another positive characteristic of ACNBs. Additionally, competitors like wasps are easily removed by managers with insecticides and the metal boxes are easily cleaned afterwards to reduce bluebird adult or nestling exposures to insecticides.

Although there were no substantial differences between the two different types of boxes, our experiment was a successful assessment of both ACNBs and WNBs because there were no detrimental effects of either box observed. While a beneficial impact of

ACNBs was predicted, a second equally interesting outcome is that at the very least they are equal in effectiveness when compared to traditional wooden boxes. Future research involving aspects such as competition for box occupancy, variation in box density, future settlement patterns, and offspring stress levels could be observed to potentially further explore any differences between these types of boxes to see if one box is viewed as higher quality over the other. The initial conclusion of this work is that for property managers this resource could provide a long-term, inexpensive resource for cavity nesting species that does not adversely affect those individuals using it.

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