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Temperature Affects Nest Box Occupancy, Nest Success, and Nestling Size in a Southeastern Population of Eastern Bluebirds (Sialia sialis)

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TEMPERATURE AFFECTS NEST BOX OCCUPANCY, NEST SUCCESS, AND NESTLING SIZE IN A SOUTHEASTERN POPULATION OF EASTERN BLUEBIRDS (*SIALIA SIALIS*)

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TEMPERATURE AFFECTS NEST BOX OCCUPANCY, NEST SUCCESS, AND NESTLING SIZE IN A SOUTHEASTERN POPULATION OF EASTERN BLUEBIRDS (*SIALIA SIALIS*)

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PREFACE: This thesis has been written in journal format and conforms to the style appropriate to my discipline. This manuscript will be submitted for publication in the Georgia Journal of Science, a peer reviewed interdisciplinary scientific journal, and therefore reflects the required formatting for this publication. Figures and tables are embedded in the text of the manuscript as required by the Georgia Journal of Science and this thesis committee.

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Chapter 1: Physiological and Behavioral Responses to Increasing Temperatures and their effects on Nest Success and Overwinter Survival in Secondary Cavity Nesting Birds

ABSTRACT

Given the critical role of temperature in the development of offspring and nest success in birds, changes in global temperatures as a result of anthropogenic climate change will likely impact the reproductive biology of avian species. Here, I examine the effects that high temperatures can have on physiology and behavior of incubating adults and nestlings during the incubation and nestling periods in secondary cavity nesting birds. Specifically, I focus on how such responses to high temperatures by both adults and nestlings can impact nest success and nestling survival. Warm temperatures during incubation can influence nest success directly by extreme temperatures causing embryonic death and indirectly through changes in incubation duration, nestling size, and adult behavior. For example, warmer nests may reduce incubation duration, thereby lessening the risk of nest predation, but, conversely, might reduce nestling size after hatching. High temperatures can also cause incubating adults to change their behavior, resulting in more trips off the nest that might further increase predation risks. Similarly, warm temperatures during the nestling period can result in smaller nestlings as a result of an energetic tradeoff between thermoregulation and growth. Adult foraging rates and foraging success also decrease as temperatures increase, reducing nestling provisioning and further contributing to reduced nestling size. Given that larger nestlings are more likely to survive to independence and have higher overwinter survival, the reduction in nestling size as a result of high temperatures may result in reduced nest success, nestling survival, and population declines in many secondary

cavity nesting bird species. Accordingly, research further examining the link between temperature and reproductive success are fundamental to avian management and conservation in the face of global climate change.

INTRODUCTION

Temperature plays a critical role in the development of offspring and reproductive success in avian species. Temperatures exceeding an optimal level during the incubation and nestling stages can affect incubation duration (Nord and Nilsson 2011; Griffith et al. 2016; Mueller et al. 2019), adult behavior (Conway and Martin 2000; Arct et al. 2022), and nestling size (Salaberria et al. 2014; Rodríguez and Barba 2016, Rodríguez et al. 2016; Andreasson et al. 2018; Mueller et al. 2019; Castro and Jones 2021), all of which have consequences on nest success and/or nestling survival. Given increasing global temperatures associated with anthropogenic climate change (Masson-Delmotte et al. 2021), understanding how temperature affects avian reproduction is essential to predict potential population-level impacts and improve future management efforts. Populations of secondary cavity nesting birds who rely heavily on artificial nest boxes may be particularly threated by high ambient temperatures as artificial nest boxes often experience more extreme and variable temperatures compared to natural cavities (Maziarz et al. 2017; Rowland et al. 2017). Here, I review how high nest temperature during the incubation and nestling stages can directly affect nest success of secondary cavity nesting species through reduced egg and nestling viability and indirectly through altered parental physiology and behavior. I also discuss how the size of fledges affects overwinter survival, an important and often overlooked factor in recruitment and population growth. For the purposes of this review, “warm” and “high” refer to temperatures exceeding the normal, ambient range experienced by a species at the specific location of those studies.

Incubation

Maintaining incubation temperatures within a narrow range (36°C - 40.5°C for most species) is essential to optimize embryonic development (Webb 1987, Conway and Martin 2000). Temperatures reaching the upper limit or exceeding this range during incubation can have direct and indirect effects on nest success, because of both physiological and behavioral responses (Figure 1).

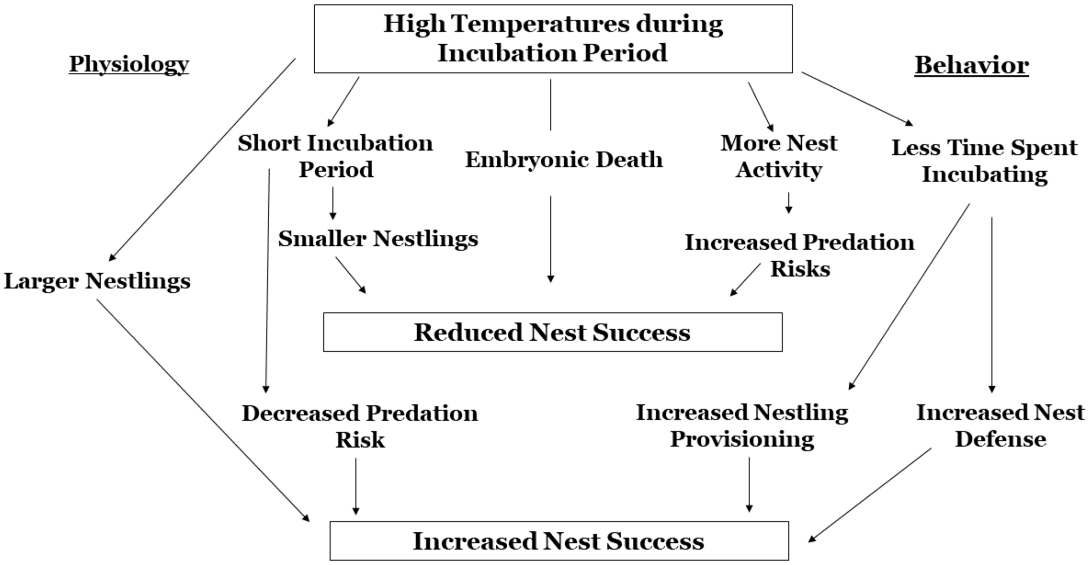

Figure 1. Flow chart depicting the physiological (right side) and behavioral (left side) effects of high temperature during the incubation period on nest success.

For example, temperatures above 40.5°C can be lethal to developing embryos, which directly reduces nest success (Webb 1987, Wilson and Tulett 1990). Nest temperatures above optimal but lower than lethal levels can also shorten the duration of embryonic development and therefore the length of the incubation period (Nord and Nilsson 2011; Griffith et al. 2016; Mueller et al. 2019; Table 1.). This shorter incubation period can be beneficial, especially in areas with high predation risk, as it reduces the overall time a nestling spends in the nest (Tombre and Erikstad, 1996; Conway and Martin 2000b;

Hepp et al. 2006). However, a shortened incubation period may come at the cost of nestling size; warmer nests can lead to smaller nestlings because of this shortened developmental period (Mueller et al. 2019; Table 1). Since larger nestlings are more likely to fledge (Andreasson et al. 2018; Bourne et al. 2020; Arct et al. 2022), warmer nest temperatures can indirectly reduce nest success by producing smaller nestlings (DuRant et al. 2013). However, in cooler environments, high temperatures can enhance developmental conditions and increase nestling size by bringing nest temperatures closer to the optimum for development in that species, thus increasing nest success (Perez et al. 2008; Arct et al. 2022; Table 1). For example, warmer nest temperatures benefitted nestling development at two high-latitude study areas that experienced cooler temperatures (Perez et al. 2008; Arct et al. 2022; Table 1).

High nest temperatures during incubation can also indirectly influence nest success through changes in adult behavior. Incubating adults in warmer nests spend less time incubating and leave the nest more frequently because they are less constrained by the energetic demands of warming eggs (Conway and Martin 2000; Arct et al. 2022; Table 1), each of which can affect nest outcomes differently (Figure 1). More frequent trips off the nest may increase the amount of activity around the nest, which increases the likelihood of detection by visually oriented nest predators, thereby reducing nest success (Conway and Martin 2000b). However, if females spend their time off nest engaged in nest defense, the warmer temperatures may reduce nest predation. Additionally, by reducing the energetic demands of incubation, warmer nest temperatures allow incubating adults to forage for longer periods of time which may increase adult body condition and enhance post-hatch nestling provisioning (Perez et al. 2008; Arct et al. 2022).

Nestling Stage

Temperatures during the nestling stage are less likely to directly reduce nest success because nestlings can thermoregulate on their own after day 2-4 (Mertens 1997; Rodríguez and Barba 2016). However, at extremely high temperatures, thermoregulation becomes insufficient and nestling body temperatures can reach lethal levels (Warriss et al. 2005; Rodríguez and Barba 2016). As in incubation, high temperatures during the nestling stage can affect physiology and behaviors in ways that indirectly affect nest success (Figure 2).

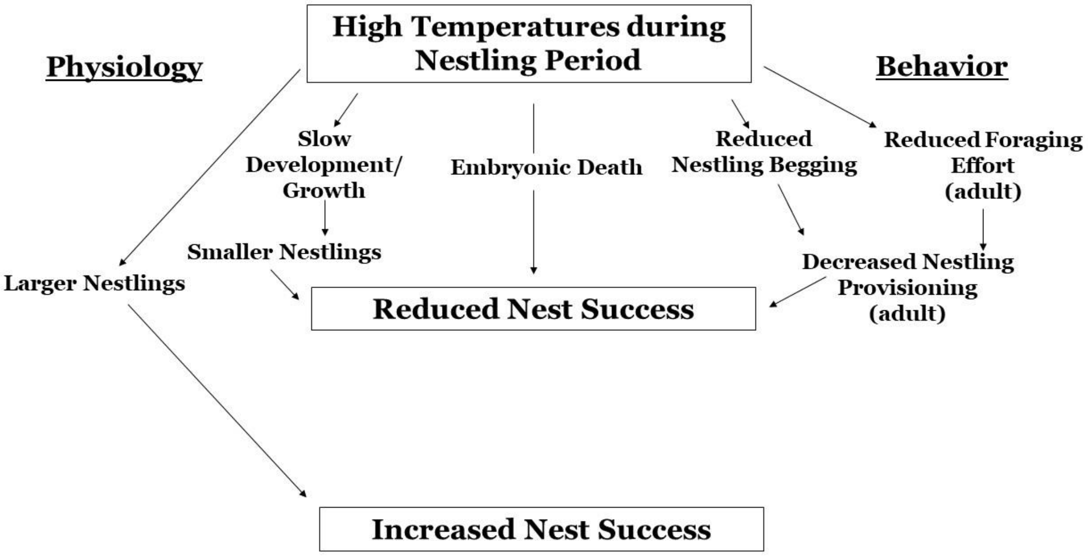

Figure 2. Flow chart depicting the physiological and behavioral effects of high temperature during the nestling period on nest success.

Indirectly, temperature is more likely to reduce nest success by causing disruptions to normal development and maturation that occur when temperatures rise outside of the optimal range (Andreasson et al. 2018; Castro and Jones 2021). Warm temperatures during the nestling period slow development, which can lead to nestlings with lower weights (Salaberria et al. 2014; Rodríguez and Barba 2016, Rodríguez et al. 2016;

Andreasson et al. 2018; Table 1) and smaller skeletal sizes (Castro and Jones 2021; Table 1). This is likely a result of a tradeoff between development and thermoregulation (Adria 2013; Rodríguez and Barba 2016; Andreasson et al. 2018); more energy must be allocated to cool the body and less is available for growth and development at warmer temperatures during the nesting period (Andreasson et al. 2018). In fact, one study reported that nests experiencing daytime temperatures that exceeded 35°C were less likely to fledge offspring (Ardia 2013; Table 1). Likewise, warmer temperatures may lead to larger nestlings in cooler, high latitude environments (Dawson et al. 2005; Table 1.) because the warmer temperatures allow nestlings to devote less energy to warming and more to growth.

High temperatures during the nestling stage can influence both nestling and adult behavior. First, nestlings beg less often in warmer nests, which may result in lower provisioning and/or a reduction in food intake and, therefore, smaller nestlings (Mueller et al. 2019; Table 1.). Adults rest more in warmer temperatures often during the warmest parts of the day, which reduces foraging rates (Funghi et al. 2019; Table 1.). As such, nestlings are fed less in warmer temperatures, leading to reduced nestling growth rates or smaller nestlings at fledge (Wiley and Ridley 2016; Andreasson et al. 2020). Given that larger nestlings are more likely to survive to independence (Andreasson et al. 2018; Bourne et al. 2020; Arct et al. 2022), any reduction in nestling body size as a result of warmer temperatures during the nestling stage will likely result in reduced nest success.

Overwinter Survival

Overwinter survival is strongly affected by nestling size; nestlings that are larger when leaving the nest are more likely to survive to the following breeding season (Perrins

1965; Garnett 1981; Both 1999; Perrins and McCleery 2001). Larger nestlings tend to have more fat reserves at fledge, which can increase likelihood of survival if nestlings experience periods of food shortages or while nestlings are learning to find food (Perrins 1965; Perrins and McCleery 2001). Larger nestlings may also have a competitive edge over smaller nestlings, which aids in competition for food resources (Garnett 1981; Perrins and McCleery 2001). Conversely, nestlings from warm nests are both smaller and exbibit lower rates of survival immediately post-fledging (Rodriguez et al. 2016) and overwinter (Greno et al. 2008). This, coupled with the fact that exposure to elevated temperatures during the incubation (Mueller et al. 2019) and nestling stages (Salaberria et al. 2014; Rodríguez and Barba 2016; Rodríguez et al. 2016; Andreasson et al. 2018) can reduce nestling and fledgling size, highlights the importance of understanding the effect of high temperatures on nestling size for overwinter survival.

CONCLUSION

Nest temperature during the incubation and nestling stages can impact nest success and nestling survival by altering embryonic development duration, adult behavior, and nestling size. As global temperatures increase as a result of climate change, these behavioral and physiological responses to temperature may increase or decrease reproductive success depending on, in part, the prevailing thermal conditions of the breeding environment of a particular species. In cooler, high latitude breeding areas, increasing temperatures may enhance reproductive success by bringing nest temperatures closer to the optimum for development in that species. However, in warmer, low latitude breeding areas where ambient temperatures regularly approach lethal levels, warming temperatures are likely to negatively affect bird populations. Behavioral and physiological responses to temperature are variable, species-specific and may be driven by local adaptations, so any changes in temperature are bound to affect the reproductive success of species in any area. Given this, research examining the unique effects of temperature on populations of cavity nesting birds is fundamental to avian management and conservation as temperatures continue to rise as a result of global climate change.

Table 1. Summary of literature examining the effects of nest box temperatures during the incubation (I) or nestling (N) periods on cavity nesting species, including study location, temperature range during study, experimental temperature increase (if applicable) and significant findings of each study.

Author	Period	Species	Location	Temperature Range (°C)	Experimental Increase (°C)	Significant Findings
Nord and Nillson 2016	I	Blue Tit	Lund, Sweden (55°42′ N, 13°28′ E)	35-38	+1.5	Length of the incubation period decreased with increased temperature
Griffith et al. 2016	I	Zebra Finch	New South Wales, Australia (31°05′ S, ‘42°42′ E)	32.1-41.5	+6.0	Artificially elevated temperature reduced hatching time by an average of 3% of the total incubation time
Mueller et al. 2019	I/N	Prothonotary warbler, Carolina wren	Millington, TN, USA (35.363°N, 90.017°W)	23.8-24.8	+1.0	Increased temperature reduced the length of the incubation and nestling periods. Reduced fledging success in prothonotary warblers. Reduced body condition in response to increased temperature in Carolina wrens. Reduced nestling begging in both species
Perez et al. 2008	I	Tree Swallow	Amherst, MA (42°22′N, 72°31′W)	18.1-25.0	+6.7	Nestlings incubated in heated nests had higher body condition and body mass
Arct et al. 2022	I	Collared flycatchers	Gotland, Sweden (57°03′ N, 18°17′ E)	14.9- 17.4	+2.5	Increased temperatures increased off bouts, reduced on-bout duration, and increased nestling mass
Rodriguez and Barba 2016	N	Great Tit	Valencia, Spain (39°42'N, 0°15'W, 30)	34-38	+4.0	Heated nestlings were lighter than controls on day 15
Rodriguez et al. 2016	N	Great Tit	Valencia, Spain (39°42'N, 0°15'W, 30)	26.4-39.8	+5.2	Heated chicks were lighter than control and cooled chicks. Estimated survival of heated fledglings was lower than that of controls
Salaberria et al. 2013	N	Spotless Starling	Madrid, Spain	-	-	Heat exposure index was negatively related to nestling body mass and wing-length

Table 1. (cont.) Summary of literature examining the effects of nest box temperatures during the incubation (I) or nestling (N) periods on cavity nesting species, including study location, temperature range during study, experimental temperature increase (if applicable) and significant findings of each study.

Author	Period	Species	Location	Temperature Range (°C)	Experimental Increase (°C)	Significant Findings
Andreasson et al. 2018	N	Blue Tit	Lund, Sweden (55°42′ N, 13°28′ E)	38 - 43.5	+5.5	Body mass gain was lower in heated nestlings compared to nestlings from control nest boxes
Adria 2013	N	Tree Swallow	Lancaster, Pennsylvania (40°01'03N, 76°17'28W).	20-40	-	Nest success declined when internal nest box temperatures exceeded 35°C.
Castro and Jones 2021	N	Great Tit	Odense, Denmark (55.372°N, 10.424°E).	34.3-36	+1.6	Nestlings in heated nest boxes were 1.6% smaller in skeletal size and 3.3% smaller in mass at fledging than those in the cooler control nests
Dawson et al. 2005	N	Tree Swallow	Prince George BC, Canada (53N, 123W)	21.7-31.8	+5.0	Offspring in heated nests had enhanced survival while in the nest and were heavier
Funghi et al. 2019	N	Zebra Finch	New South Wales, Australia (13.100S, 17.400E)	17-44	-	As temperatures exceeded 35 C, heat dissipation behaviors increased and foraging effort was reduced

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TEMPERATURE AFFECTS NEST BOX OCCUPANCY, NEST SUCCESS, AND NESTLING SIZE IN A SOUTHEASTERN POPULATION OF EASTERN BLUEBIRDS (*SIALIA SIALIS)*

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Given the critical role that temperature plays in avian reproductive biology, rising temperatures as a result of global climate change will likely impact reproductive success of many bird populations. Secondary cavity nesting birds, many of which rely on artificial nest boxes to maintain population levels, may be particularly at risk because increased temperatures inside nest boxes can determine whether nest boxes are selected, reduce nest success, and/or negatively affect the growth and survival of offspring. We examined the effect of nest box color on nest box temperature and the influence of nest box temperature, nest box color, entrance orientation, and nest site features (distance to forest cover, distance to water) on nest box occupancy, nest success, and nestling size at fledge in Eastern Bluebirds at a restored grassland in central Georgia. From March-August 2020-2022, we monitored temperature inside 50 nest boxes, recorded nest outcome, and measured nestling size (tarsus). We compared the effect of nest box color on nest box temperatures using an ANOVA and used Akaike's Information Criterion (AICc) to determine which variables were most strongly associated with nest box occupancy, nest success, and nestling size at fledge. White painted nest boxes had significantly cooler average daily high, average daily lows, and average daily temperatures than control nest boxes. However, Eastern Bluebird females selected boxes with warmer daily lows, daily highs, and daily averages. We posit that this allowed the female to reduce energy expenditure during incubation and shorten the nesting period. Nest success was strongly associated with year, likely because of increased nest predation driven by the warmer ambient temperatures experienced during the 2022 breeding season. Unlike occupancy, cooler nest boxes were associated with increased nest success, possibly due to cooler boxes decreasing trips off the nest by incubating female and thus reducing predation risks. Nestlings that fledged from painted nest boxes, nest boxes with cooler average daily high temperatures, and nests initiated during 2021 were larger. This is likely due to a tradeoff whereby nestlings that don’t have to devote energy to cooling down their bodies can instead devote that energy to growth. These results suggest there may be an optimal temperature range where nest box temperature maximizes reproductive fitness. Determining the optimal temperature range for occupancy, success, and nestling growth may yield conflicting results, but can help target management to ensure specific goals are met.

Keywords: temperature, nest box, reproductive success, conservation, avian ecology, eastern bluebird, occupancy

INTRODUCTION

Surface temperatures are rising due to anthropogenic climate change, with current models predicting an increase in global temperature of 1.6°C by the year 2040 (Masson-Delmotte et al. 2021). Consequences of rising surface temperatures, including increased daily temperatures, greater temperature variability, and more extreme temperatures, are already affecting many ecosystems and will become more pronounced in the coming decades (Masson-Delmotte et al. 2021). Because temperature plays a critical role in the incubation, development, and survival of avian species (Hepp et al. 2015), global temperature changes will affect birds throughout their nesting cycle, from nest site selection to overwinter fledgling survival. For example, even small temperature increases can shorten the incubation and nestling periods (Rodríguez and Barba 2016; Mueller et al. 2019), lower the quality of nestling body condition (Rodríguez and Barba 2016; Mueller et al. 2019; Andreasson et al. 2018; Castro and Jones 2021), and reduce post-fledging survival in some species (Nord and Nilsson 2016; Rodríguez et al. 2016; Greño 2018). High nest temperatures have also been associated with declines in overall nest success (Ardia 2013). If human-driven climate change proceeds as currently predicted, we're likely to observe continued declines in population size of many wild bird species.

Secondary cavity nesting birds rely on natural holes or abandoned cavities previously excavated by primary cavity nesters. Given their inability to create their own nesting cavities, the breeding densities of secondary cavity nesting species can be limited by the availability of suitable nesting sites (Newton 1994), and suboptimal temperatures can deter females from selecting sites (Blem and Blem 1996; Adria et al. 2006). Artificial nest boxes can provide additional nesting opportunities and have increased population sizes in some species, such as the Wood Duck (Hegge 1991). However, artificial nest boxes experience more variable and extreme temperatures compared to natural cavities (Maziarz et al. 2017; Rowland et al. 2017). This may lower reproductive success and reduce survival due to the increased energetic demands of nestlings at extreme high and low temperatures (Conway and Martin 2000).

Altering characteristics of nest box design and location may be an effective strategy to mitigate extreme and variable temperatures in artificial cavities. For example, painting the exterior highly reflective colors can lower internal nest box temperatures (Griffiths et al. 2017) because darker colored nest boxes absorb more solar radiation compared to lighter colored boxes (Nussear et al. 2000). Nest boxes with openings facing west may also provide cooler internal nest box microclimates (Ardia et al. 2006; Butler et al. 2009). Similarly, placing boxes closer to canopy cover may reduce nest box temperatures from increased shade cover (Wachob 1996; DuRant et al. 2013). Therefore, supplying nest boxes that provide temperatures that are optimal for nest site selection should increase occupancy and, thus, population size (Newton 1994). However, nest box color (Browne 2010), nest box entrance orientation (Navara and Anderson 2011) and/or the position of nest boxes relative to water (Milligan and Dickinson 2016) and edge habitat (Rendell and Robertson 1990) have also been documented to influence occupancy independent of nest temperature and are also important features to consider when implementing thermally considerate nest box designs.

Temperature can also affect nest success through physiological and/or behavioral mechanisms. Adults with warmer nests can leave the nest more frequently because they are less constrained by the energetic demands of warming eggs (Conway and Martin 2000; Arct et al. 2022). This increases the amount of activity around the nest and could increase the likelihood of detection by visually oriented nest predators, thereby reducing nest success (Conway and Martin 2000b). Conversely, higher temperatures may reduce nest predation (and thus increase nest success) if incubating adults can devote more of their daily energetic budget on nest defense. In extreme cases, elevated nest temperatures may cause egg or nestling death (Webb 1987; Sloane et al. 2022). Like occupancy, nest box characteristics such as proximity of nest box to site features (i.e. water, forest edge; Patton 1994; Allen and Stumpf 2021) and entrance orientation (Butler et al. 2019), may also influence nest success.

Finally, nest box temperatures may affect the size of a nestling at fledge. For example, nestlings from warmer nest boxes may be smaller because they must devote more energy to thermoregulation as opposed to devoting it to growth (Salaberria et al. 2014; Rodríguez and Barba 2016; Rodríguez et al. 2016; Castro and Jones 2021). Given that body size at fledge is a strong predictor of adult survival (Tinbergen and Boerlijst 1990; Both 1999; Greño et. al 2008; Rodríguez et al. 2016b), increased nest temperatures may have negative population level consequences if nestlings fledge at smaller sizes as temperatures increase (Rodríguez et al. 2016b; Castro and Jones 2021).

Eastern Bluebirds (*Sialia sialis*) are secondary cavity nesting thrushes that occupy a wide range of habitats including grasslands, meadows, and other natural areas with open areas surrounded by forest-edge habitat (Gowaty and Plissner 2020). While Bluebird populations are currently not of major conservation concern, their regular use of artificial nest boxes and multiple broods over a long breeding season make them an excellent study species to investigate the effects of nest box temperatures on reproductive success. Here, we first examine the effect of nest box color (treatment) on nest box temperature. Next, we examine the influence of nest box temperature, treatment, entrance orientation, and nest site features (distance to forest cover, distance to water) on nest box occupancy, nest success, and nestling size at fledge in a southeastern population of Eastern Bluebirds.

MATERIALS & METHODS

Study Site

We conducted this study over three breeding seasons (March through August) from 2020 to 2022 at a 180-acre restored grassland at Panola Mountain State Park (PANO, Figure 1), in the Piedmont province of north central Georgia, USA. The grassland consists of primarily warm-season grasses bordered by mixed hardwoods and surrounded to the north, east, and west by a large stream.

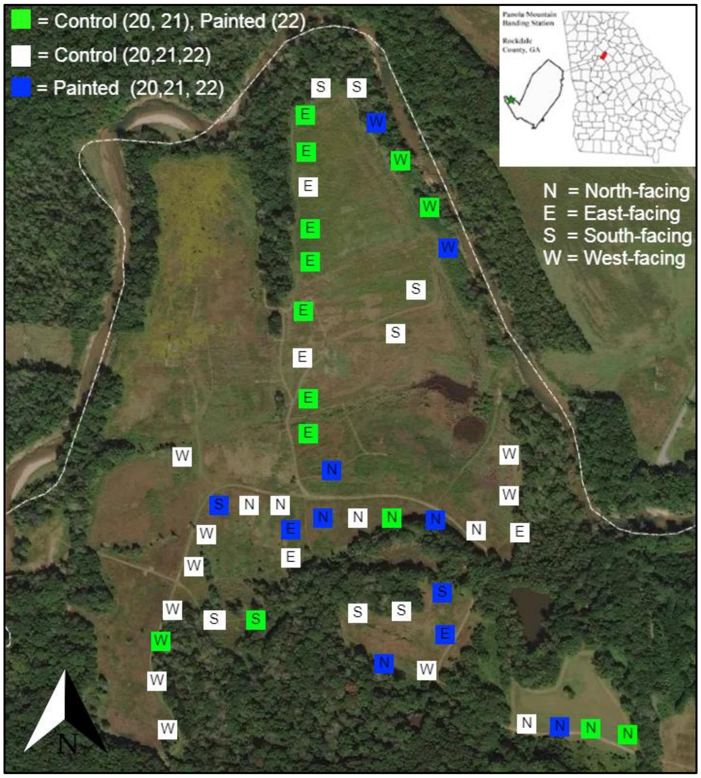

Figure 1: Map of Eastern Bluebird nest box locations at Panola Mountain State Park in Central Georgia (inset). Treatments during each year are indicated with colors (green squares indicate control boxes during 2020, 2021 and painted during 2022, white square indicates control boxes during all years, and blue squares indicate boxes that were painted during all years). Entrance orientations of individual nest boxes are indicated by letters (N: North, E: East, S: South, W: West).

Nest Boxes

The Panola Mountain Bluebird Network, a volunteer organization collecting nest box breeding data within the park, installed a series of 50 standard, side-opening bluebird nest boxes throughout the grassland between 2014 and 2020. Volunteers mounted the nest boxes on posts approximately 5 feet from the ground throughout the study area, primary along vehicle access paths (Figure 1). Prior to the start of the 2020 breeding season, we painted the exterior of 11 nest boxes with low VOC white exterior grade paint and painted 14 more (for a total of 25) prior to the 2022 breeding season (Figure 1). Within each treatment, we stratified entrance orientation across the four cardinal directions (N, S, E, W, Figure 1). At the conclusion of the season, we estimated the distance from forest edge and distance from water for each nest box using Google Earth (2022).

Nest Box Temperature

Prior to the start of each breeding season, we installed iButton data loggers (Thermochron iButton, Maxim Inc.) on the interior sidewall of each nest box and programmed them to record temperature every 60 minutes. We summarized temperatures using 3 averages: average daily high (the average high temperature across the 24-hourly recordings), average daily low (the average low temperature across the 24-hourly recordings), and overall average (a simple average of all 24-hourly readings). For occupancy analyses, we calculated each temperature average from March 28th through June 24th (inclusive dates that iButtons were operational across all three seasons; 85% of nesting attempts fell within this date range). For nest success and nestling size analyses, we calculated each average during active nesting days, from the initiation of incubation (back-calculated from the first egg hatch date) to the date of fledge or predation (estimated as the median date between nest check visits documenting occupied then empty nests).

Nest Box Occupancy and Success

We visited nest boxes every 3-4 days to determine occupancy (defined as the presence of at least one egg), record the number and age of eggs and/or nestlings, take nestling measurements (see below), and determine nest success. We recorded nest success for each nesting attempt as either successful (at least one fledge) or not successful (no fledges). We assumed nestlings to have successfully fledged if they had vacated the nest at or beyond 13 days of age, at which point they are fully feathered and capable of short-distance flight (Gowaty and Plissner 2020). After the breeding season was complete, we determined the ordinal start date of each occupied nest by back-calculating from the day on which first egg was recorded.

Nestling size

We individually identified nestlings by banding each bird with a uniquely numbered U.S. Fish and Wildlife Service band between 5-8 days, the point at which a metal band is unlikely to affect fledging success (Pinkowski 1975). We used tarsus length (mm) to determine nestling size at fledge, as it is a good indicator of adult size and limits variability seen in measures of nestling mass (Rising and Somers 1989). We used the last measurement recorded on or beyond nestling age of 10 days, because tarsus growth stabilized (slope of linear regression, Table 1) after day 10 and reflects tarsus size at fledge for our dataset.

Table 1: Calculated slopes of regression for all tarsus measures between various age ranges. Dashed line indicates age range at which slope stabilized.

Nestling Age Range (days)	Slope
7-15	0.5030104
8-15	0.456543
9-15	0.4581868
10-15	0.3314389
11-15	0.3311556
12-15	0.2917984
13-15	0.3322979
14-15	0.1580065

Statistical analysis

We compared the effect of treatment on nest box temperatures using an ANOVA. We used an information-theoretic approach (Akaike's Information Criterion corrected for small sample sizes [AIC_c]; Burnham and Anderson 2002) to determine the effect of nest box characteristics (treatment, nest box temperature during nesting, entrance orientation, distance to forest edge, distance to water) on nest box occupancy, nesting attempt success, and nestling tarsus size at fledge. To account for annual and seasonal variations, we included year and ordinal start date in all models for the nest success and nestling size analyses. We looked at 22 models of occupancy and 26 models of nest success and nestling size, plus the null and global (all variables) models (Table 2). Models with $\Delta AIC_c \leq 4.0$ (top models) were considered to have the most support; models not meeting this criterion were excluded from further analyses. If there were multiple top models, we calculated model-averaged parameter estimates, odds ratios, and 95% confidence intervals (CI) to determine the relative influence of each characteristic in the candidate set of models (Burman and Anderson 2002).

Table 2. All AIC models used in analysis for nest box occupancy, nest success, and nestling size at fledge for nest boxes and nestlings between March-August from 2020-2022 at Panola Mountain State Park in central GA.

Occupancy Models	Nest Success, Nestling Size Models
AH+AL+YR	AH+AL+AT+DW+DC+TR+OR+YR+OD
AH+DC+YR	AH+AL+AT+TR+YR+OD
AH+DW+YR	AH+AL+YR+OD
AH+YR	AH+DC+YR+OD
AL+DC+YR	AH+YR+OD
AL+YR	AL+DC+YR+OD
AT+AH+AL+YR	AL+YR+OD
AT+YR	AT+AH +AL+YR+OD
DC+YR	AT+DW+YR+OD
DW+DC+OR+YR	AT+YR+OD
DW+DC+YR	DC+YR+OD
DW+YR	DW+DC+OR+YR+OD
Null	DW+DC+YR+OD
OR+YR	DW+YR+OD
TR+AL+DC+YR	Null
TR+AT+AH+AL+DW+DC+OR+YR	OR+YR+OD
TR+DC+YR	TR+AH+YR+OD
TR+DW+DC+OR+YR	TR+AL+YR+OD
TR+DW+YR	TR+AT+YR+OD
TR+OR+YR	TR+DC+YR+OD
TR+YR	TR+DW+DC+YR+OD
YR	TR+DW+YR+OD
	TR+OR +DW +DC+YR+OD
	TR+OR+YR+OD
	TR+YR+OD
	YR+OD

AH: Average daily high nest box temperature (C^{o}), AL: Average daily low nest box temperature (C^{o}), AT: Average nest box temperature (C^{o}), DC: Distance from forest cover (m), DW: Distance from water (m), YR: Year (2020, 2021, 2022), OD: Start date (Ordinal dates), TR: Nest box treatment (paint, control), OR: Nest box entrance orientation (N, E, S, W)

RESULTS

Nest Box Temperature

Painted nest boxes had significantly cooler average daily highs, daily averages, and average daily lows than control nest boxes (32.4^{o}C, 24.2^{o}C, and 17.1 ^{o}C in painted boxes; 34.7, 25.2, 17.6 in control boxes; *F*=26.97, *F*=16.40, *F*=21.24 respectively, *P*<0.005 for all three; Figure 2, Table 2). Notably, painted boxes had an average daily high that was 4.2°C cooler than control boxes during the 2022 breeding season, the largest temperature difference across years ($P<0.005$; Table 3).

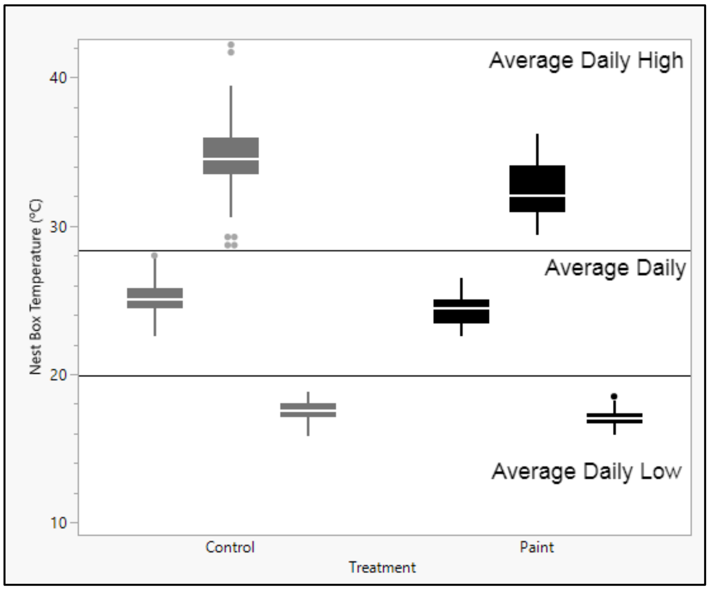

Figure 2: Internal temperature (°C) of painted (black) and control (dark grey) nest boxes across three temperature categories: Average daily high (top), average daily (middle), and averaged daily low (bottom). Temperatures were measured between March 28th and June 24th from 2020-2022 at Panola Mountain State Park.

Table 3. Nest box temperatures (°C) inside control and painted nest boxes between March 28th and June 24th from 2020-2022 in nest boxes at Panola Mountain State Park in central Georgia. Asterisks indicate significant differences ($P<0.05$) in temperature between treatment groups within the same year. Bolded values indicate temperatures that were significantly different ($P<0.05$) between 2022 and 2020/2021.

Year	Treatment	Average Daily Temperature (°C)	Average Daily High Temperature (°C)	Average Daily Low Temperature (°C)
2020	Control	24.6 ± 0.13*	33.6 ± 0.32*	17.4 ± 0.11*
	Paint	23.1 ± 0.11*	30.7 ± 0.24*	16.8 ± 0.19*
2021	Control	24.7 ± 0.12*	33.6 ± 0.27*	17.5 ± 0.01
	Paint	23.4 ± 0.09*	30.9 ± 0.24*	17.1 ± 0.16
2022	Control	**26.8 ± 0.16***	**37.8 ± 0.44***	17.9 ± 0.14*
	Paint	**25.0 ± 0.10***	**33.6 ± 0.30***	17.4 ± 0.12*
All Years	Control	25.2 ± 0.13*	34.7 ± 0.27*	17.6 ± 0.07*
	Paint	24.2 ± 0.15*	32.4 ± 0.28*	17.1 ± 0.09*

Nest Box Occupancy

Approximately 58.7% of the 150 nest boxes available during the combined years were occupied at least once (Table 4). Of the 103 control boxes, 59.2% were occupied with a total of 357 eggs and 234 nestlings (Table 4, Table 5). Of the 47 painted boxes, 57.4% were occupied with a total of 149 eggs and 91 nestlings (Table 4, Table 5). Six occupancy models were in the top models (ΔAICc ≤4.0) and had a combined ω_i of 0.75 (Table 6). Model-averaging revealed three characteristics where confidence intervals of the odds ratio did not overlap one (Table 7). Nest boxes with warmer average daily high temperatures, warmer average daily low temperatures, and warmer average daily temperatures were more likely to be occupied (Figure 3).

Table 4. Percent occupancy and nest success for painted and control nest boxes from 2020-2022 at Panola Mountain State Park.

Year	Treatment	Occupancy (#occupied/#available)	Nest Success (#succesful/#attempts)
2020	Control	66.7% (26/39)	52.9% (18/34)
	Paint	36.4% (4/11)	0.00% (0/5)
	Total	60.0% (30/50)	46.2% (18/39)
2021	Control	43.6% (17/39)	68.0% (17/25)
	Paint	63.6% (7/11)	54.5% (6/11)
	Total	48% (24/50)	63.9% (23/36)
2022	Control	72.0% (18/25)	24.0% (6/25)
	Paint	64.0% (16/25)	25.0% (5/20)
	Total	68.0% (34/50)	24.4% (11/45)
All Years	Control	59.2% (61/103)	48.8% (41/84)
	Paint	57.4% (27/47)	30.6% (11/36)
	Total	58.7% (88/150)	43.3% (52/120)

Table 5. Precent eggs hatched, precent eggs fledged, and precent nestlings fledged from painted and control nest boxes between March-August from 2020-2022 at Panola Mountain State Park.

Year	Treatment	%hatched (#nestlings/#eggs)	%eggs fledged (#fledges/#eggs)	%nestlings fledged (#fledges/#nestlings)
2020	Control	75.7% (109/144)	41% (59/144)	54.1% (59/109)
	Paint	56.5% (13/23)	0% (0/23)	0% (0/13)
	Total	73.1% (122/167)	35.3% (59/167)	48.4% (59/122)
2021	Control	72.5% (79/109)	54.1% (59/109)	74.7% (59/79)
	Paint	74% (37/50)	50% (25/50)	67.6% (25/37)
	Total	73% (116/159)	52.8% (84/159)	72.4% (84/116)
2022	Control	44.2% (46/104)	16.3% (17/104)	37.0% (17/46)
	Paint	53.9% (41/76)	25% (19/76)	46.3% (19/41)
	Total	48.3% (87/180)	20% (36/180)	41% (36/87)
All Years	Control	65.5% (234/357)	37.8% (135/357)	57.7 % (135/234)
	Paint	61.1% (91/149)	29.5% (44/149)	48.4% (44/91)
	Total	64.2% (325/506)	35.4% (179/506)	55.1 % (179/325)

Table 6: AIC model results on nest occupancy between March-August from 2020-2022 in nest boxes at Panola Mountain State Park in central Georgia. Only models with ΔAICc ≤ 4.0 are shown. Null and global models are included for reference.

Model[1]	k[2]	AICc[3]	ΔAICc[4]	ω_i[5]
AL+YR	3	181.88	0	0.22
AL+DC+YR	4	182.57	0.69	0.15
DW+YR	3	183.20	1.32	0.11
AH+AL+YR	4	183.49	1.61	0.10
AT+AH+AL+YR	5	183.76	1.88	0.085
DW+DC+YR	4	183.81	1.93	0.082
Null	1	187.61	5.73	0.012
Global	11	188.29	6.41	0.0088

[1] AH: Average daily high nest box temperature (C°), AL: Average daily low nest box temperature (C°), AT: Average nest box temperature (C°), DC: Distance from forest cover (m), DW: Distance from water (m), YR: Year (2020, 2021, 2022)

[2] Number of parameters

[3] Akaike information criterion corrected for small sample sizes

[4] Difference between AICc values of current model and most supported model

[5] Relative support for a model out of the candidate set

Table 7: Model averaged parameter estimates ($\hat{\beta}$), unconditional variances (Var), and odds ratios (95% CI) for nest box occupancy computed across top models (6; Burnham and Anderson 2002). Bolded text indicates characteristics in which the confidence intervals of the odds ratio does not overlap one.

Characteristic[1]	$\hat{\beta}$ (Var)	Odds ratio (CI)
AH	**-0.15 (0.0073)**	**0.861 (0.87, 0.85)**
AL	**0.86 (0.23)**	**2.36 (3.71, 1.51)**
AT	**0.51 (0.0048)**	**1.67 (1.68, 1.65)**
DC	-0.012 (0.00038)	0.988 (1.00, 0.99)
DW	0.0051 (0.00013)	1.01 (1.01, 1.00)
YR[2021-2020]	-0.49 (0.47)	0.613 (1.54, 0.243)
YR[2022-2021]	0.80 (0.45)	2.23 (5.37, 0.921)

[1] AH: Average daily high nest box temperature (C°), AL: Average daily low nest box temperature (C°), AT: Average nest box temperature (C°), DC: Distance from forest cover (m), DW: Distance from water (m), YR: Year (2020, 2021, 2022)

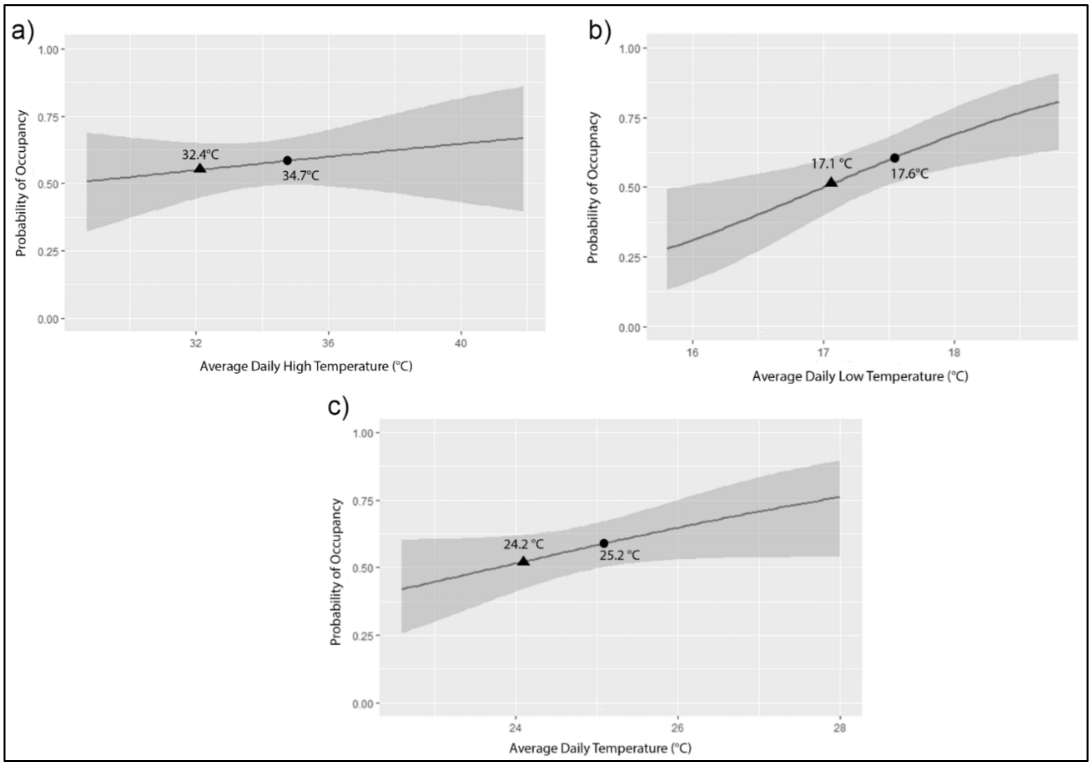

Figure 3: Estimated probability and 95% confidence interval (in grey) of nest box occupancy as a function of a) average daily high temperature (°C) b) average daily low temperature, and c) average daily temperature between March 28th and June 24th from 2020-2022 in Eastern Bluebird Panola Mountain State Park in central Georgia. Average daily high temperatures of treatment groups (Triangle: painted, Circle: control) are indicated on the probability curve.

Nest Success

Approximately 43.3% of nesting attempts were successful across the three study years, 48.8% of control boxes and 30.6% of painted boxes (Table 4). One hundred and thirty-five nestlings fledged from 84 attempts in control boxes and 44 nestlings fledged from 36 attempts in painted boxes (Table 4, Table 5). Six models of nest attempt success were in the top models (ΔAICc ≤4.0) and had a combined ω_i of 0.76 (Table 8). Model-averaging revealed four characteristics where confidence intervals of the odds ratio did not overlap one (Table 9). Nest boxes used during the 2021 breeding season and those with cooler daily high temperatures, cooler daily low temperature, and cooler daily temperatures were more likely to be successful (Figure 4).

Table 8. AIC model results on nest success at Panola Mountain State Park from March-August from 2020-2022. Only models with ΔAICc ≤ 4.0 are shown. Null and global models are included for reference.

Model[1]	k[2]	AICc[3]	ΔAICc[4]	ω_i[5]
AL+YR+OD	4	103.78	0	0.30
AL+DC+YR+OD	4	105.55	1.77	0.12
AT+YR+OD	4	106.01	2.23	0.10
AH+AL+YR+OD	4	106.05	2.27	0.09
TR+AL+YR+OD	4	106.11	2.22	0.09
YR+OD	3	107.09	3.31	0.06
Null	1	113.96	10.18	0.0018
Global	12	120.47	16.69	0.000070

[1] AH: Average daily high nest box temperature (C°), AT: Average nest box temperature (C°), AL: Average daily low nest box temperature (C°), DC: Distance from forest cover (m), TR: Nest box treatment (paint, control), OD: Start date (Ordinal dates), YR: Year (2020, 2021, 2022)

[2] Parameter of each model

[3] Akaike information criterion corrected for small sample sizes

[4] Difference between AICc values of current model and most supported model

[5] Relative support for a model out of the candidate set

Table 9: Model averaged parameter estimates ($\hat{\beta}$), unconditional variances (Var), and odds ratios (95% CI) for nest success computed across top models (6; Burnham and Anderson 2002). Bolded text indicates characteristics in which the confidence intervals of the odds ratio does not overlap one.

Characteristic[1]	$\hat{\beta}$ (Var)	Odds ratio (CI)
AH	**-0.027 (0.0017)**	**1.027 (1.03, 1.02)**
AT	**-0.25 (0.0024)**	**0.78 (0.78, 0.78)**
AL	**-0.30 (0.09)**	**0.74 (0.88, 0.62)**
DC	0.0036	1.00 (1.00, 1.00)
OD	0.039 (0.017)	1.04 (1.86, 1.00)
TR	0.018 (0.0049)	1.018 (1.03, 1.00)
YR[2021-2020]	-1.03 (0.65)	0.36 (1.28, 0.010)
YR[2022-2021]	**2.27 (0.60)**	**9.68 (31.3, 2.99)**

[1] AH: Average daily high nest box temperature (C°), AT: Average nest box temperature (C°), AL: Average daily low nest box temperature (C°), DC: Distance from forest cover (m), OD: Start date (Ordinal dates), TR: Nest box treatment (paint, control), YR: Year (2020, 2021, 2022)

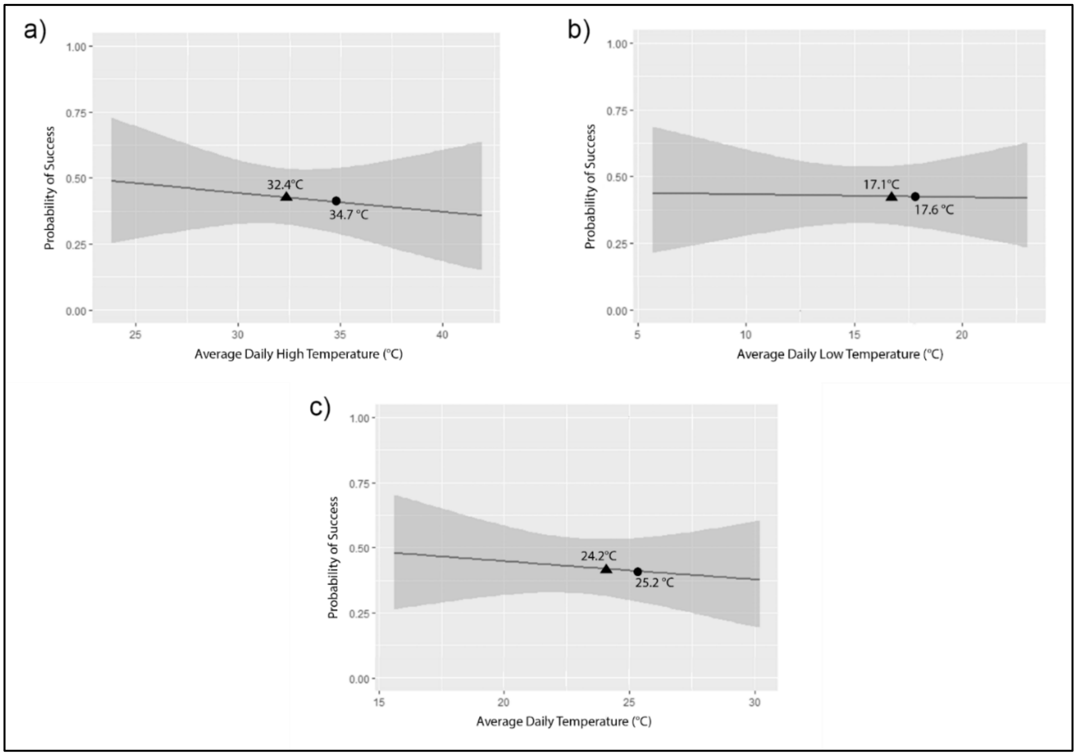

Figure 4: Estimated probability and 95% confidence interval (in grey) of nest success as a function of a) average daily high temperature (°C) b) average daily low temperature (°C) and c) average daily temperature (°C) during nesting attempts from 2020-2022 in nest boxes at Panola Mountain State Park in central Georgia.

Nestling Size

We measured tarsus size at fledge for 106 of 179 fledges. Three models of nestling tarsus size at fledge were in the top models (ΔAICc ≤4.0) and had a combined ω_i of 0.66 (Table 10). Model-averaging revealed three characteristics where confidence intervals of the odds ratio did not overlap one (Table 11). Larger nestlings were associated with nest boxes that had cooler average daily high temperatures (Figure 5), were painted white (Table 12), and were reared during the 2021 breeding season.

Table 10. AIC model results on tarsus length (mm) at fledge (n=106) at Panola Mountain State Park from March-August from 2020-2022. Only models with ΔAICc ≤ 4.0 are shown. Null and global models are included for reference.

Model[1]	k[2]	AICc[3]	ΔAICc[4]	ω_i[5]
TR+DC+YR+OD	4	364.01	0	0.38
AH+DC+YR+OD	4	365.75	1.74	0.16
TR+DW+DC+YR+OD	6	366.25	2.24	0.12
Global	12	372.64	8.63	0.0051
Null	1	379.89	15.88	0.00013

[1] AH: Average daily high nest box temperature (C°), DC: Distance from forest cover (m), DW: Distance from water (m), OD: Start date (Ordinal dates), TR: Nest box treatment (paint, control), YR: Year (2020, 2021, 2022)

[2] Parameter of each model

[3] Akaike information criterion corrected for small sample sizes

[4] Difference between AICc values of current model and most supported model

[5] Relative likelihood that a model is the best model

Table 11: Model averaged parameter estimates ($\hat{\beta}$), unconditional variances (Var), and odds ratios (95% CI) for tarsus at fledge computed across top models (3; Burnham and Anderson 2002). Bolded text indicates characteristics in which the confidence intervals of the odds ratio does not overlap one.

Characteristic[1]	$\hat{\beta}$ (Var)	Odds ratio (CI)
AH	**-0.11 (0.0012)**	**0.90 (0.90, 0.89)**
DC	-0.0070 (0.0011)	99 (1.01, 1.00)
DW	-0.00040 (0.0070)	1.00 (1.00, 1.00)
OD	-0.0058 (0.0019)	0.994 (1.00, 0.99)
TR	**-0.41 (0.039)**	**0.66 (0.72, 0.60)**
YR[2021-2020]	0.35 (0.16)	1.15 (1.94, 1.00)
YR[2022-2021]	**-1.28 (0.15)**	**0.28 (0.38, 0.20)**

[1] AH: Average daily high nest box temperature (C°), DC: Distance from forest cover (m), DW: Distance from water (m), OD: Start date (Ordinal dates), TR: Nest box treatment (paint, control), YR: Year (2020, 2021, 2022)

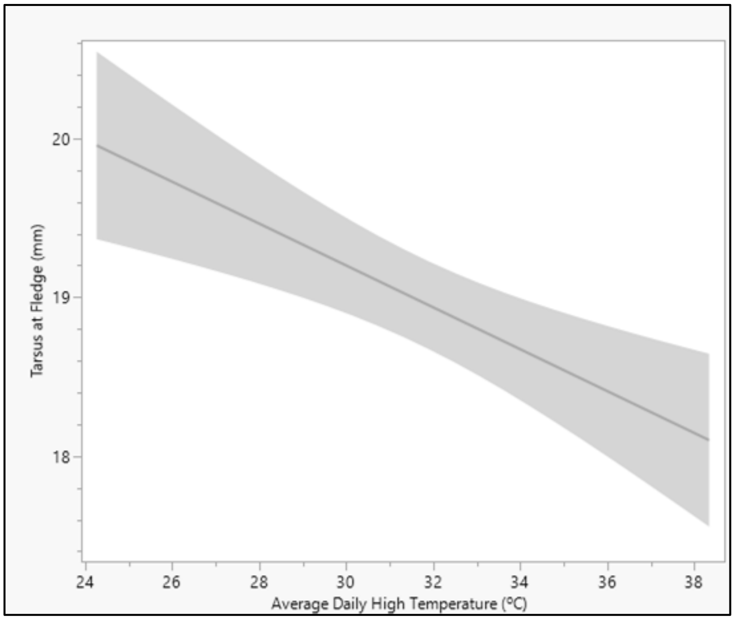

Figure 5: Linear regression with 95% confidence intervals (gray) of tarsus at fledge (mm) as a function of average daily high temperature (°C) between March-August from 2020-2022 at Panola Mountain State Park in central GA.

Table 12. Average tarsus length (mm) for nestlings reared in painted and control nest boxes at Panola Mountain State Park in central Georgia from March-August from 2020-2022.

Year	Treatment	Average Tarsus Length (mm)
2020	Control	19.26
	Paint	-
	Combined	19.26
2021	Control	19.13
	Paint	19.94
	Combined	19.38
2022	Control	17.61
	Paint	18.75
	Combined	18.34
All Years	Control	19.05
	Paint	19.45
	Combined	19.16

DISCUSSION

Nest Box Occupancy

While the application of white paint to the exterior of nest boxes is a cheap and effective way to reduce temperatures within nest boxes, it was not associated with nest occupancy or nest success. In central Georgia, where ambient temperatures often exceed the lethal temperature for developing embryos, we expected that Eastern Bluebirds would select cooler nest boxes, however occupancy was more likely in nest boxes with warmer temperatures (Figure 3). Other studies have documented selection of warmer nest boxes in cavity nesting species (Blem and Blem 1995; Adria et al. 2006), possibly because warmer temperatures reduce the energetic demands of incubation and can allow incubating females to expend energy on other activities such as foraging and nestling provisioning (Perez et al. 2008; Arct et al. 2022). This shift in daily energy expenditure can lead to larger females and offspring, increasing the likelihood of overwinter survival (Arct et al. 2022). Warmer nest boxes may also lead to faster nestling growth rates

(Dawson et al. 2005) and require shorter incubation and nestling periods (Mueller et al. 2019). This, coupled with the high predation rates we witnessed at PANO (unpubl. data), may drive the selection of warmer nest boxes to reduce overall nesting time and thus, exposure to predation. Interestingly, we saw no association between nest box treatment and nest box occupation even though painted boxes were 4.1% cooler than control boxes, on average (Table 3). While this does follow the pattern of greater occupancy in warmer nest boxes (Blem and Blem 1995; Adria et al. 2006), this temperature difference corresponds to only a small increase (approximately 5%) in predicted occupancy (Figure 3).

Nest Success

Annual variation appears to play the strongest role in influencing Eastern Bluebird nest success; 63.9% of nests initiated during the 2021 breeding season were successful, whereas only 24.4% of nests were in 2022 (Table 4). The reduced nest success experienced in 2022 may be explained by increased nest predation driven by warmer ambient temperatures. Nest predation rates by snakes and birds increase as daily maximum temperatures increase (Cox et al. 2013), and though we can't say with certainty that the failures were caused by predation, we did note more snake activity, finding snakes inside of occupied nest boxes on three separate occasions during the warmer, less successful 2022 breeding season. Cooler nest boxes were also associated with increased nest success (Figure 4), a trend that has been documented in Tree Swallows, another secondary cavity nesting species (Ardia 2013), possibly due to changes in female incubation behavior reducing predation risks. Cooler nest box temperatures can reduce the frequency of trips off the nest by incubating females (Arct et al. 2022), which may lessen the activity around the nest and potentially reduce detection by nest predators. Interestingly, treatment was not associated with nest success despite the cooler temperatures in painted nest boxes. It is likely that the differences in average temperatures between painted and control nest boxes in our study (1.0 °C, Figure 4) was not large enough to influence female incubation behavior and subsequently nest success. Currently, changes in female incubation behavior as a result of experimentally manipulated nest temperatures have only been documented occurring at average temperature differences above 2.5°C (Bryan et al. 1999; Ardia et. al 2009; Arct et al. 2022).

Nestling Size

Our data show that a 2°C increase in the average daily high nest temperature corresponds to an approximately 0.5mm reduction in tarsus length at fledge (Figure 5), results that are congruent with other studies (Salaberria et al. 2014; Rodríguez and Barba 2016; Rodríguez et al. 2016). Nestlings from boxes experiencing greater daily high temperatures are likely spending more energy cooling down their elevated body temperatures, which reduces the amount of energy that can be allocated for growth and can lead to lower food intake (Geraert et al. 1996; Rodríguez et al. 2016; Andreasson et al. 2018), leading to smaller nestlings. As expected, tarsi of nestlings from cooler, painted boxes were 2.1% larger than nestlings from warmer, control boxes (Painted = 19.45mm, Control = 19.05mm; Table 7). Likewise, tarsi of nestlings reared during the cooler 2021 breeding season were 5.7% larger at fledge compared to those reared in warmer 2022 breeding season (2021 = 19.38mm, 2022 = 18.34mm; Table 12). Fledging larger nestlings may have population level-consequences as larger nestlings are more likely to survive the winter

(Greño 2008; Rodríguez et al. 2016; Rodríguez et al. 2016b) and be recruited into the breeding population the following year (Both et al.1999).

Conclusion

Our overall findings highlight the mixed fitness effects of nest box temperature on reproductive success at various stages in the nesting cycle in Eastern Bluebirds. On one hand, warmer nest boxes have apparent benefits; warmer nest boxes are more likely to be occupied and may reduce female energy expenditure leading to increased body condition. On the other hand, warmer nest boxes may lead to behavioral changes that attract nest predators and reduce nestling size at fledge and therefore post-fledging survival. These results indicate there may be an optimal temperature range where nest box temperature maximizes reproductive fitness. Given that global temperatures are projected to rise over the upcoming decades, determining what this temperature range is through experimental manipulations of temperatures in nest boxes will allow the implementation of nest box designs that maximize development and reproductive success and mitigate population level consequences of climate change.

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