Biology Honors Thesis

Thermal stress in artificial cavity-nesting Eastern Bluebirds: killing them with kindness?

William J. Zahran

May 2018

APPROVED BY:

Director of Thesis: ________________
Susan B. McRae

Faculty assessor: ________________
Christopher N. Balakrishnan

Director of Undergraduate Studies: ________________
Jean-Luc Scemama

On this day, May 1, 2018

THERMAL STRESS IN ARTIFICIAL CAVITY-NESTING EASTERN BLUEBIRDS: KILLING THEM WITH KINDNESS?

by

William J. Zahran

A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

By

William J. Zahran

Greenville, NC

May 2, 2018

Approved by:
Susan B. McRae, Ph.D
Department of Biology, College of Arts and Sciences

Thermal stress in artificial cavity-nesting Eastern Bluebirds: killing them with kindness?

William J. Zahran

A thesis submitted to the Department of Biology, East Carolina University, in partial fulfillment
of the requirements for Biology Honors Thesis

Advisor: Susan B. McRae, Ph.D
East Carolina University
Department of Biology

May 1, 2018

I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has it been submitted elsewhere as coursework for this or another degree.

Signed:______________________________ Date: 5/1/2018
William J. Zahran

Thermal stress in artificial cavity-nesting Eastern Bluebirds: killing them with kindness?

William J. Zahran

Department of Biology, East Carolina University, Greenville N.C. 27858-4353, U.S.A.

ABSTRACT - Artificial nest boxes are used to help cavity-nesting species with declining populations that compete for limited nest sites. Proper use of nest boxes has been shown to lower predation rates and ectoparasite loads. However, most nest boxes do not provide the insulation benefits of natural cavities and may be increasing thermal stress on cavity-nesting species. Recent studies have found evidence for the effects of extreme ambient temperatures on hatching and fledging success in passerines. Therefore, I hypothesized that clutches experiencing the highest mean temperatures would have a greater likelihood of hatching failure. I further expected that broods exposed to high mean temperatures during the nestling period would influence nestling growth and survival rates. I studied multiple-brooded Eastern Bluebirds breeding in nest boxes at ECU's West Research Campus. I placed programmable thermochron iButtons in a consistent position within nest boxes to measure temperature at 10-minute intervals continuously during the incubation and nestling periods. I recorded data on hatching success, nestling size, and fledging success. I found that high mean temperatures during incubation significantly impacted the proportion of unhatched eggs in a nest. Yet, some late broods tolerated prolonged temperatures above 35º C where boxes remained in full sun because no natural shading was available. My results suggested that some nest boxes reached temperatures above a tolerable threshold for some bluebird embryos to survive. To mitigate the effects of heat stress, nest boxes should be designed with insulation properties or placed where they receive natural shading to provide a stable microclimate for cavity-nesting passerines.

Acknowledgments

I would like to sincerely thank the people and organizations that made this project possible: my lab mates in the McRae and Balakrishnan labs, my parents, Stephen and Carolyn Zahran, the Department of Biology, the Honors College, and East Carolina University. I would like to especially thank my mentor, Dr. Susan B. McRae, for her knowledge, patience, and guidance and for allowing me to be a part of the Bluebird Project. My project was supported by the Honors College.

Table of Contents

List of Tables

List of Figures

Introduction

Ambient temperature during incubation and nestling stages is a critical factor in the growth and survival of passerine birds because nestlings have not fully developed thermoregulatory ability (Murphy 1985, Rodriguez & Barba 2016, Greño et al. 2008). Suboptimal temperatures during the incubation and nestling periods are associated with decreased growth and survival in small birds (Rodriguez & Barba 2016, Larson et al. 2015). Rising temperatures in the southeastern United States due to global climate change may pose or exacerbate threats to passerines (Conrey et al. 2016, Gleick et al. 2010). Higher than normal temperatures during incubation have been shown to negatively affect hatching success (Reyna & Burggren 2017). Heat exposure has also been shown to negatively impact body size, thus reducing fitness (Salaberria et al. 2014). There is an urgent need to investigate the effects of temperature on reproductive success in small passerines.

Nest boxes have allowed researchers to access cavity-nesting species for observation and manipulation (Lambrechts et al. 2012, Larson et al. 2018). Early studies of passerines showed that artificially placed nest boxes have a number of benefits when compared to natural cavities including lower predation rates and lower ectoparasite loads (Nilsson 1984, Robertson & Rendell 1990, Kuitunen & Alekonis 1992, Tomás et al. 2007). Nest boxes also provide additional nest sites where deforestation and habitat destruction have decreased the availability of nest sites (Libois et al. 2012, Catry et al. 2011, Larson et al. 2015). As a result, nest boxes have been used as a conservation tool for a variety of cavity-nesting species (Larson et al. 2018). However, studies are just beginning to consider the importance of other parameters for successful nest boxes besides predator avoidance (Lloyd & Martin, 2004). Factors like microclimate within the nesting cavity and nest box design have been shown to affect survival and reproduction in avian species (Lloyd & Martin 2004, Amat-Valero et al. 2014).

Design and placement are important in mitigating the effects of heat exposure within a nest box (Pinowski et al. 2006, Lambrechts et al. 2012). Not all species have higher reproductive success in nest boxes compared to natural cavities (Purcell et al. 1997). Tree cavities have relatively stable temperatures, which may be optimal for reproductive success (Wiebe 2001). By contrast, wooden nest boxes have a more variable microclimate, and as a result, can put more stress on nesting species (Amat-Valero et al. 2014, Larson et al. 2018). Microclimate can also vary among nearby nest boxes based on placement in relation to the sun (Butler et al. 2009). Abnormally high temperatures within the nest cavity require greater energy expenditure by the nestlings to conserve water, thus negatively affecting survival through dehydration (Amat & Masero 2003). This research sought to determine if high temperatures inside the nest box are negatively affecting the reproductive success of Eastern Bluebirds (*Sialia sialis*).

This study focused on a resident population of Eastern Bluebirds in Greenville, North Carolina. The Eastern Bluebird (hereafter, 'bluebird') is a multiple-brooded species that readily nests in artificial nest boxes, thus making them a model species for observation (Gowaty & Plissner 1998). Populations of bluebirds can be used for studies ranging from ecology and evolution to behavior and genetics (Gowaty & Plissner 1998). Faced with increasing temperatures, bluebirds reared in nest boxes that are not optimized to mitigate the effects of temperature may experience decreased survival (Dawson et al. 2005). Therefore, there is a need to investigate the maximum temperature bluebird embryos and nestlings can withstand for prolonged periods of time and how nest boxes may be optimized to improve microclimate conditions.

The objective of this study was to determine whether hatching success and/or nestling condition were negatively impacted by high temperature inside the nest box. I measured the temperature inside nest boxes used by breeding bluebirds during the incubation and nestling periods. I predicted that nest boxes experiencing the highest maximum temperatures inside the box during incubation would have lower rates of reproductive success due to higher rates of hatching failure. I further predicted that broods exposed to higher maximum temperatures would have, on average, smaller chicks at fledging.

Methods

This research was part of long-term study of Eastern Bluebirds nesting in purpose-built boxes placed at East Carolina University's West Research Campus (35°37'N, 77°28'W) in Greenville, North Carolina, USA. The natural area included in West Research Campus is a 202 hectare mixed habitat containing scrub, restored wetlands, and open fields managed by East Carolina University by mowing and prescribed burns. This study analyzed data collected during the 2014 and 2017 breeding seasons. Methods for setting up and monitoring nest boxes were the same for both years.

In each year, thirty nest boxes (Bailey's Home for Bluebirds; Figure 1) were monitored at the site and placed at distances at least 50 meters apart because bluebirds prefer not to nest within sight of another pair. Nest boxes were installed ~1.5 m above the ground on an aluminum conduit pole each with a conical steel predator baffle painted black. Nest boxes were made of 1.9 cm soft wood with a particle board roof covered with sheet metal and measuring 14.2 cm wide, 15.2 cm deep, and 27.7 cm high. Nest boxes had an entrance hole diameter of 4.2 cm to exclude larger species and surrounded by sheet metal to prevent enlargement. The front panel opened to allow access for monitoring.

The nest boxes were set up in early-March and monitored through August until the final brood fledged each year. Nest boxes were checked at least every three days and at least three times per week throughout the breeding season. At each nest check, I described any new nest material and recorded nest height, the number of eggs and whether they were warm, and the number of chicks and their approximate developmental stage. Observations of adult behavior and sightings were also recorded to include individual identities, presence of male or female, and reaction to researcher checking the nest box. On the tenth day after the day the first chick in the

Figure 1. Nest box design used in this study. The entrance hole diameter measures 4.2 cm. Each air hole diameter measures 1.6 cm. Total height of the nest box measures 30.3 cm.

brood hatched (Day 0), each chick's tarsus length and longest pin feather length were measured using calipers (±0.1 mm). Each chick's weight was measured using a Pesola (±0.2 g). Reproductive success was measured using hatching success (number of eggs hatched/clutch size) and fledging success (number of chicks surviving to fledging/brood size). Nestling condition was estimated using weight/tarsus (mean weight/tarsus length per nest).

Temperature data were collected using Thermochron iButtons (Embedded Data Systems, DS1921G-2048) at most active nests. One iButton was placed at each active nest box that recorded temperature throughout incubation and nestling periods until the brood fledged. iButtons were attached to the inside door of nest boxes at nest height using Velcro to measure the temperature to which the birds were exposed inside the nest boxes. iButtons were programmed to collect a temperature measurement every 10 minutes simultaneously. iButtons were swapped out every two weeks at active nests due to their limited storage capacity. In 2014, differences in procedures included that the iButton inside the nest box was placed on the ceiling of the nest box protected by a thin mesh netting, and that iButtons were programmed to record temperature data every five minutes.

Statistical analyses were performed used JMP (Version 12, SAS Inc.). Figures were created using Tableau (Version 10.4, Tableau Software). Dates were converted into Julian dates (January 1 = 1) for all analyses. The effects of temperature were analyzed using generalized mixed models in JMP v12.0 with year and nest attempt identity as random factors.

Results

In the 2014 and 2017 breeding seasons, there were 47 and 40 nesting attempts, respectively. From among these nests, temperature data were collected from 22 nests between May 31st and August 10th in 2014, and 30 nests between May 20th and July 29th in 2017 (N_{total}=52). From among these 52 nests, temperature data were obtained for only the incubation period in 7 nests, only the nestling period in 7 nests, and both incubation and nestling periods in 38 nests. The reproductive success data were summarized in Table 1. I recorded temperature data during these time periods based on the days first eggs were laid. Nests that experienced predation (N=4), hatching failure due to desiccation or misshapen eggs (N=3), or parental desertion (N=1) were excluded from this study.

To determine whether temperatures increased in the latter half of the breeding season, I compared the mean daily temperature, mean maximum daily temperature, and mean minimum daily temperature of early broods with a first egg date in April or May and late broods with a first egg date in June or July to determine the difference in temperatures throughout the breeding season (Table 2). Mean temperature was calculated based on daily temperatures a nest experienced from the beginning of the iButton deployment until the brood fledged. The mean maximum daily temperature was calculated from the daily maximum temperatures experienced in each nest box every 24 hours from the beginning of iButton deployment until the brood fledged. The mean minimum daily temperature was calculated the same way using daily minimum temperatures. Mean temperature (F_5=0.17, $P<0.05$) and mean maximum temperature (F_5=0.18, $P<0.05$) were significantly different between early and late broods in 2014.

Since only in 2017 did I measure nest box temperatures across the entire breeding season, I related these data to hatching rates during this year only. Based on a generalized linear mixed

Table 1. Summary of breeding data for 2014 and 2017 based on the first egg date.

Year	April-May Mean Hatching Success Rate (number of eggs hatched/clutch size)	June-July Mean Hatching Success Rate (number of eggs hatched/clutch size)	April-May Mean chick weight/tarsus length (± SE)	June-July Mean chick weight/tarsus length (± SE)	April-May Mean Fledging Success Rate (arcsine square root chicks survived to fledge/brood size) (± SE)	June-July Mean Fledging Success Rate (arcsine square root chicks survived to fledge/brood size) (± SE)
2014	0.58 ± 0.17	0.59 ± 0.09	1.19 ± 0.05	1.13 ± 0.02	0.75 ± 0.25	0.73 ± 0.12
2017	0.79 ± 0.048	0.56 ± 0.10	1.22 ± 0.04	1.17 ± 0.04	0.84 ± 0.08	0.7 ± 0.15

Table 2. Summary of temperature data measurements across nest boxes in 2014 and 2017 based on first egg date.

Year	April-May Mean Temperature (°C ± SE)	June-July Mean Temperature (°C ± SE)	April-May Mean Maximum Temperature (°C ± SE)	June-July Mean Maximum Temperature (°C ± SE)	April-May Mean Minimum Temperature (°C ± SE)	June-July Mean Minimum Temperature (°C ± SE)
2014	27.27 ± 0.20*	27.20 ± 0.33*	37.12 ± 0.44*	36.00 ± 0.57*	19.94 ± 0.28	20.67 ± 0.30
2017	26.61 ± 0.32	29.11 ± 0.35	34.94 ± 0.43	36.94 ± 0.37	19.53 ± 0.42	22.90 ± 0.43

*Significant difference between early broods and late broods in the same year ($P<0.05$).

model, hatching rate declined significantly over the season in 2017 ($F_{1,22.1}$=6.50, P=0.02). Mean nest box temperatures taken across the site increased significantly from May through July in 2017 (Figure 2).

The mean temperatures inside the nest box ($F_{1,51.0}$=0.48, P=0.49), hatching success ($F_{1,51.0}$=1.49, P=0.23), and fledging success rate ($F_{1,46.0}$=0.03, P=0.87) did not differ significantly between 2014 and 2017. Data from both seasons were therefore pooled for subsequent analyses.

Seasonal temperature increase

First egg date was the most accurate parameter for measuring the timing of nest initiation according to the nest monitoring schedule. First egg date was therefore used to represent the relative timing of each nesting attempt over the breeding season. To determine whether mean nest box temperature changed significantly over the course of the season, I used a general linear mixed model combining 2014 and 2017 data. The fixed effect on mean nest box temperature was the first egg date, and the random effects were box number and year. I found a significant increase in mean temperature during the nesting period in relation to the date the first egg was laid ($F_{1,37.3}$=4.24, $P<0.05$; Figure 3). This held true in spite of the fact that there was a period of time late in the 2014 nesting season when mean daily temperatures were temporarily cooler.

Seasonal hatching success

Of the 52 nesting attempts investigated, 32.7% of nests had 1 unhatched egg, 26.9% nests had 2 unhatched eggs, and in 5 nests none of the eggs hatched. Since it is not uncommon to find one unhatched egg in passerine nests (SBM, pers. comm.), I focused on cases where 2 or more eggs failed. There was compelling evidence to suggest that heat may have factored in the demise of at least some of these eggs. Thirteen (65%) of the 20 nests with 2 or more unhatched eggs had a first egg date in June or July. Four of the 5 nests (80%) with complete hatching

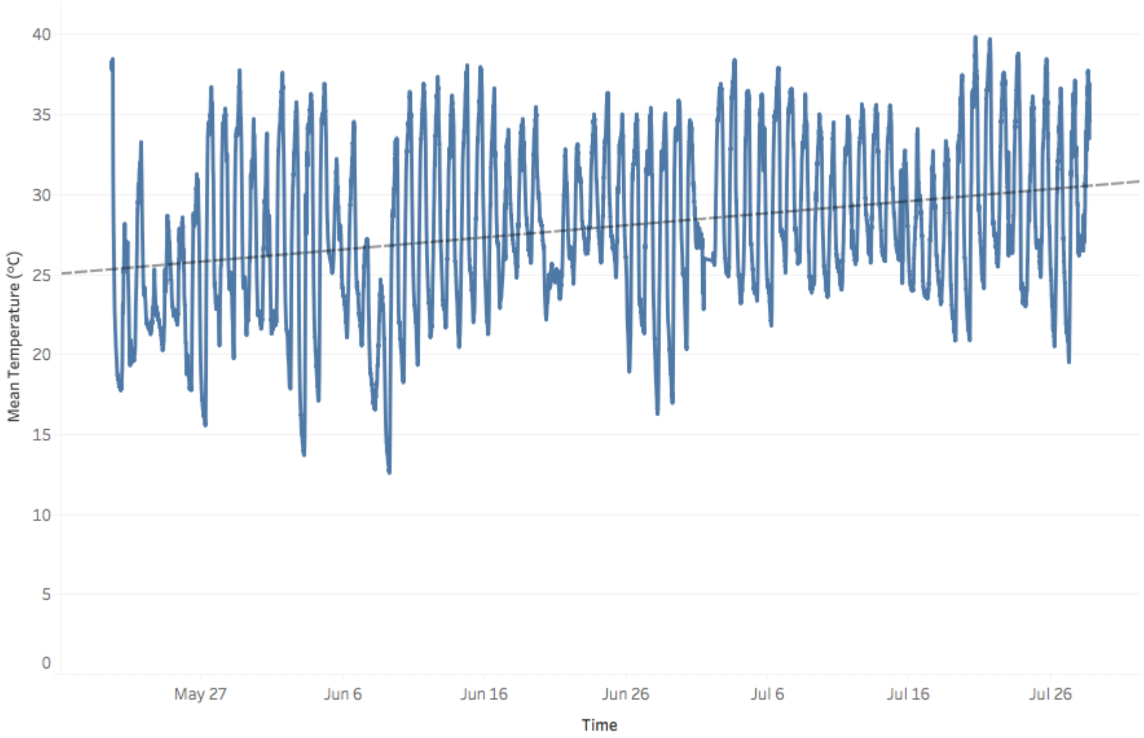

Figure 2. Seasonal variation in mean nest box temperature. Mean nest box temperature taken at 10-minute intervals across all the nest boxes is based on N=3-12 simultaneously deployed iButtons ($r^2=0.08$, $P<0.0001$).

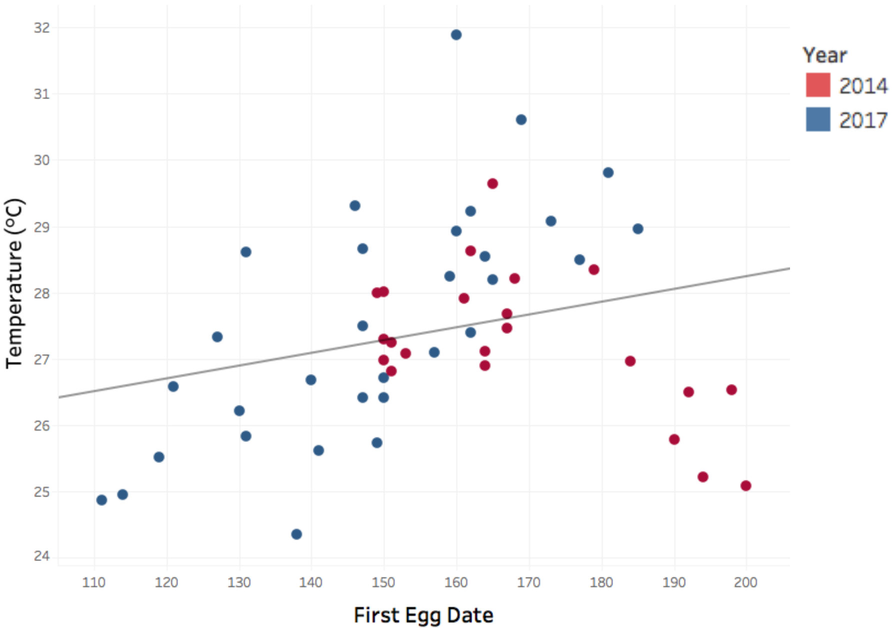

Figure 3. Mean daily nest box temperature in relation to first egg date. Julian date is used where Day 1 = January 1. Each point represents the mean daily temperature across the entire iButton deployment for a single nesting attempt ($F_{1,37.3}$=4.24, $P<0.05$).

failure, nests had a first egg date in June or July. To investigate the hatching success rate throughout the breeding season, I compared the first egg date to the proportion of eggs in a clutch that hatched. The proportions of hatched eggs were normalized using the arcsine of the square root of the proportion. The proportion of hatched eggs declined significantly over the breeding season in relation to the first egg date ($F_{1,51.0}=29.97$, $P<0.001$; Figure 4).

In one case in 2014, three unhatched eggs out of a clutch of four eggs were collected after only 1 egg hatched on the 12th day of incubation. The eggs were opened and analyzed to determine which stage in development the embryos were. Each embryo was in a different stage of development. Based on size and appearance, the first embryo was estimated to be approximately one third developed. The other two eggs had even smaller embryos, as though developed to a point one and two days earlier, respectively. Based on the first egg date, incubation time, and hatch date, the egg that hatched was likely the first egg laid in the clutch.

Does nestling condition decline over the season?

In passerine birds, nestling condition is usually measured using weight as a function of tarsus length (Martínez-de la Puente et al. 2010). I investigated the relative condition of nestlings among nests over the breeding season by comparing the first egg date to the mean weight/tarsus measurement of each brood. Measures were taken on all nestlings in the brood ten days after the day the first nestling in the brood hatched. Mean weight/tarsus length did not significantly decline over the breeding season ($F_{1,0.3}=0.55$, $P=0.75$; Figure 5). This held true even when excluding the four broods that were banded on Day 10 and 1 brood that was banded on Day 12. Brood reduction of one or two chicks in brood sizes of three or more occurred in four nests prior to Day 9.

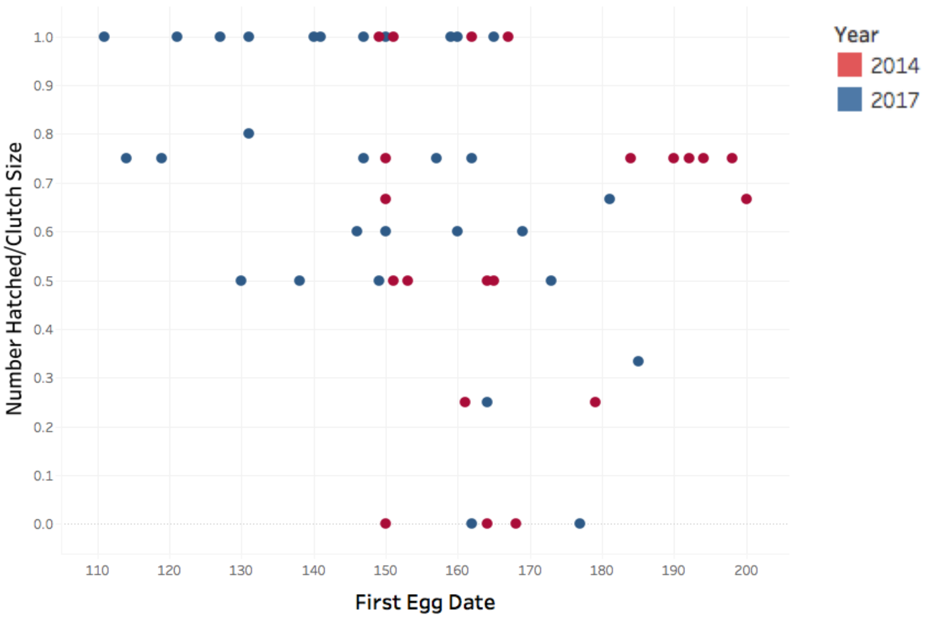

Figure 4. Seasonal decline in hatching success. Each point represents the hatching success in one brood related to the first egg date of the brood (F=4.65, P=0.04). This analysis excludes 3 nests where mortality occurred due to desiccation or desertion. Julian date is used where Day 1 = January 1.

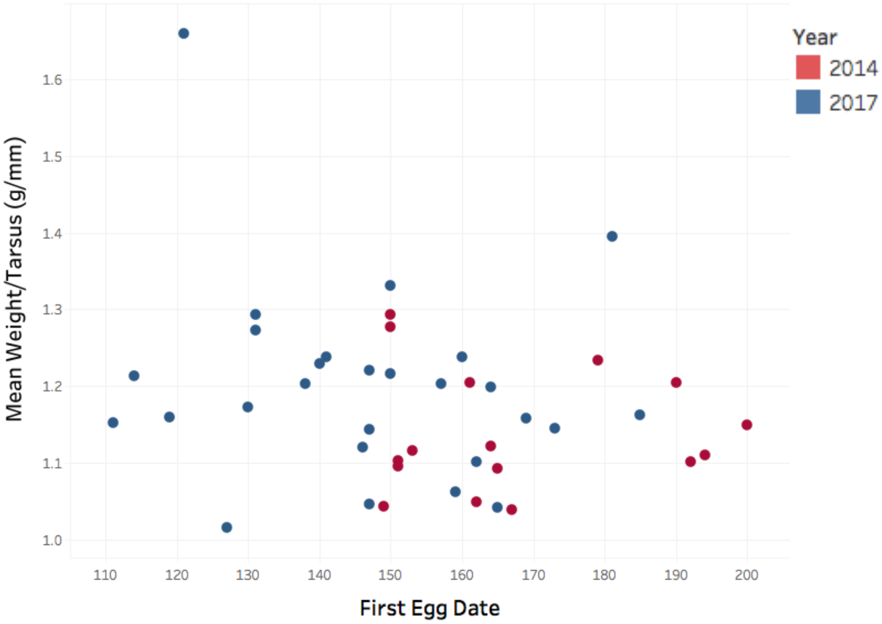

Figure 5. Mean weight/tarsus length in relation to first egg date. Each point represents the mean weight/tarsus measurement for one nesting attempt (F=0.55, P>0.05). Julian date is used where Day 1 = January 1.

Did fledging success decline over the season?

To investigate seasonal effects on fledging success, I compared first egg dates to the proportion of chicks that survived to fledge successfully. Fledging success was normalized using the arcsine of the square root of the proportion of fledged chicks/brood size. A generalized mixed model comparing first egg data and fledging success showed a nonsignificant relationship ($F_{1,46.0}<0.001$, $P>0.9$).

Effects of temperature during the incubation period

To investigate the effects of temperature, I separated the temperature experienced between the incubation period and nestling period. First, I set out to determine if seasonal increase in mean nest box temperature could have contributed to the decline in hatching success rate directly due to heat stress on embryos. For the incubation period, I included for each nest all temperature data collected from the first egg date until the date the first chick hatched. To determine if high temperatures during incubation impacted the proportion of eggs hatched, I used generalized mixed models to compare mean daily maximum temperature and maximum temperature during incubation to the hatching success. The highest maximum temperature observed during incubation across the site was 45.5 °C, and the lowest maximum temperature observed during incubation across the site was 35.5 °C. The mean daily maximum temperature during incubation did not significantly impact the hatching success rate ($F_{1,2.6}=1.48$, $P=0.32$; Figure 6). Maximum nest temperatures (highest temperature reached) during incubation also did not significantly impact the hatching success rate ($F_{1,30.0}=0.32$, $P=0.57$).

I also investigated the effects of longer durations of high temperatures during incubation on hatching success using a generalized mixed model. The number of hours a nest sustained temperatures above 35 °C during incubation had a significant effect on hatching success

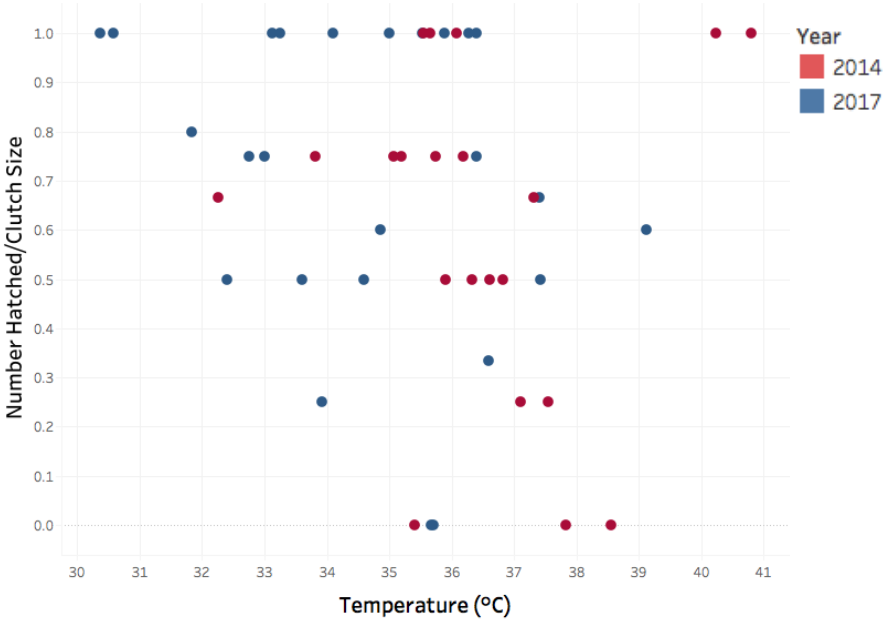

Figure 6. Hatching success was not significantly related to mean maximum daily nest box temperature during incubation. Each point represents the proportion of eggs hatched in one nest related to the mean daily maximum temperature in the nest box during incubation ($F_{1,2.6}$=1.48, P=0.32).

($F_{1,33.9} = 5.47$, $P = 0.02$; Figure 7). However, the number of hours a nest sustained above 38 °C during incubation did not have a significant effect on hatching success ($F_{1,33.9} = 1.46$, $P = 0.23$).

Effects of temperature during the post-hatching period

To determine if high temperatures impacted nestling condition, I compared mean daily maximum temperatures experienced in the nest box to the weight/tarsus measurement. The temperature analyzed for the nestling period included all data collected from the date the first chick hatched until the date the last chick in the brood fledged. A generalized mixed model controlling for measures of several chicks in each brood revealed that the mean maximum daily temperature during the entire nesting period did not have a significant effect on the mean nestling condition ($F_{1,21.0}=0.15$, $P=0.70$). The mean maximum daily temperature during the nestling stage did not have a significant effect on the mean weight/tarsus length ($F_{1,23.8}=0.01$, $P=0.92$). Brood reduction occurred in four of these nests prior to Day 9.

I investigated the effects of high temperatures on fledging success by comparing mean maximum temperatures and the proportion of the brood that fledged. The mean maximum daily temperature during the entire nesting period did not impact the fledging success rate ($F_{1,46.0}=0.10$, $P=0.75$). The mean maximum temperature during the nestling period did not impact the fledging success rate ($F_{1,43.0}=0.14$, $P=0.71$).

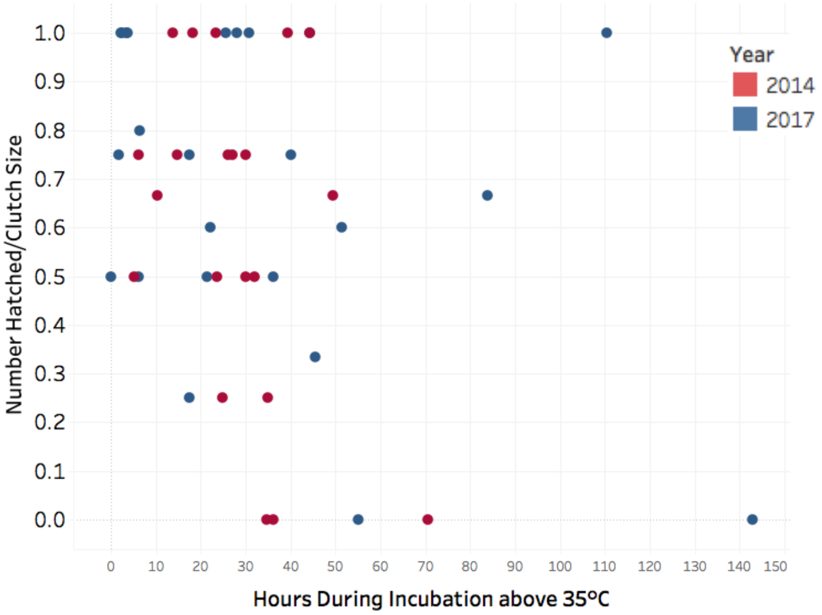

Figure 7. The effect of sustained hours during incubation above 35 °C on hatching success. Each point represents the proportion of eggs hatched in one nest related to the number of hours the nest sustained temperatures above 35 °C during incubation ($F_{1,33.9} = 5.47$, $P = 0.02$).

Discussion

My findings suggest that high nest box temperatures may impact the reproductive success of some nesting bluebird pairs. In addition, my results suggest that not only higher temperatures, but also the duration of those high temperatures, could affect reproductive success. The data indicate a relationship between temperature and hatching success, but they do not indicate a relationship between temperature and nestling condition or fledging success. Faced with a changing climate, bluebirds may be vulnerable to rising temperatures predicted worldwide (Meehl et al. 2000).

The effects of temperature can be complex to measure as they may be direct or indirect (Salaberria et al. 2014). Species that lay several clutches each year endure a range of temperatures throughout a long breeding season (Burton 2006). Therefore, plasticity in maternal investment in nests over the course of a season based on the prevailing conditions may explain some decline in reproductive success and may be important in adaptation to climate change (Bleu et al. 2017). My study only analyzed temperature inside the nest box, rather than the clutch or the brood directly, so I can only discuss the direct effects of temperature experienced by the eggs and nestlings excluding known causes of embryo or nestling mortality. Because the focus of the study was high temperature, and it is difficult to imagine how parent bluebirds could reduce temperature of their offspring much through contact in a cavity nest, I feel justified in assuming that the temperature within the box approximated the temperature experienced. These are important considerations for studies examining multiple-brooded species that breed over the course of a long season with high variation in temperature.

As in other parts of their range, bluebirds here readily used nest boxes. At the West Research Campus field site, bluebirds are limited by nest sites as evidenced by nearly full occupancy during the last seven breeding seasons. Temperature inside the nest boxes across the site increased significantly over the season simultaneous with a significant decrease in hatching success. This finding suggested that higher temperatures may be impacting reproductive success at the egg stage and required a closer investigation. The mean maximum daily temperature represented the highest temperatures a nest box endured during incubation and can reveal the effects of high temperatures on hatching success (Cunningham et al. 2013). However, my results did not indicate a significant effect of mean maximum daily temperature on hatching success. While some nests did experience complete hatching failure at the highest mean maximum daily temperatures, some nests had 100% hatching success. This variation in survival under the same environmental conditions may be explained by genetic variation between broods (Bateson et al. 2016). Specifically, recent work on heat-shock-proteins has shown that some individuals may be more tolerant of extreme temperatures due to genetic predisposition (Vinoth et al. 2018).

A maximum temperature was not reached that caused complete mortality across the site making it difficult to determine whether there was a developmentally-dependent thermal threshold for survival. It is likely that a temporal component of temperature impacts survival (Hurley et al. 2018). My analysis of the duration of high temperatures suggest that some embryos may be able to tolerate high temperature spikes but not for a prolonged period of time. The duration of temperature sustained above 35 °C had a significant negative impact on hatching success. However, this relationship was not observed when analyzing time sustained above 38 °C in nest boxes. These findings could indicate that clutches that are adapted to survive the highest temperatures are not experiencing mortality. However, clutches that were sensitive to high temperatures experienced mortality when exposed to temperatures above 35 °C for a prolonged period.

Cases in both 2014 and 2017 provide anecdotal evidence of heat stress negatively impacting hatching success. In one case in 2014 where three of four embryos in a clutch perished, the developmental stages of the embryos were clearly observable. The embryo that survived was likely the first egg laid and was therefore further along in development than the other embryos. This finding suggests that the three embryos perished at the same time early in development due to an event such as an environmental extreme that halted their development. The embryo that survived may have been past a critical stage in development allowing it to survive the extreme environmental condition. In 2017, a remarkable number of late broods in June and July had greater than two unhatched eggs with four nests in that time period experiencing 100% hatching failure. Together, these results suggest high temperatures could be approaching the limits of tolerance of at least some individuals at the embryonic stage.

I did not detect a relationship between any temperature measures and nestling condition or fledging success. Temperatures are likely not reaching maximums high enough to negatively impact nestlings directly. However, this study did not measure survival after fledging. Body condition at fledging can affect survival and is a reflection of conditions during the nestling stage (Greño et al. 2008). Further, brood reduction observed in this population may allow parents to have some reproductive success in response to increasing temperatures (Vedder et al. 2018).

Mean annual temperatures are forecasted to continue rising as a result of climate change (Gleick et al. 2010) and will likely cause new and exacerbated threats to passerines along with the continued decline of available nest sites. It is imperative that researchers continue to monitor temperature in artificial cavities and its effects on species during the breeding season. It is likely that current nest box designs will need to be improved in the near future to mitigate the effects of extreme temperatures. The nest boxes used in this study have some qualities that make them better equipped to insulate eggs and nestlings from high temperatures, including holes for aeration and thick, dense wood. Recent work by Larson et al. (2018) has explored materials and designs to improve the microclimate in artificial nest boxes. Their recommendations may need to be considered for this population of bluebirds if temperature continues to negatively impact reproductive success. Additionally, nest box placement is important to allow natural shading that protects nest boxes from prolonged sun exposure whilst still being in a sufficiently open environment to favor colonization by bluebirds over other cavity-nesters.

Literature cited

Amat, J. A. & Masero, J. A. (2003). Predation risk on incubating adults constrains the choice of thermally favourable nest sites in a plover. *Animal Behaviour*, 26, 293-300.

Amat-Valero, M., Calero-Torralbo, M. A., Vaclav, R., & Valera, F. (2014). Cavity types and microclimate: implications for ecological, evolutionary, and conservation studies. *Int. Journal of Biometeoro.l*, 58, 1983-1994.

Ambrosini, R., Ferrari, R. P., Martinelli, R., Romano, M., & Saino, N. (2005). Seasonal, meteorological, and microhabitat effects on breeding success and offspring phenotype in barn swallow, *Hirundo rustica*. *Ecoscience*, 13, 298-307.

Bateson, Z. W., Hammerly, S. C., Johnson, J. A., Morrow, M. E., Whittingham, L. A., & Dunn, P. O. (2016). Specific alleles at immune genes, rather than genome-wide heterozygosity, are related to immunity and survival in the critically endangered attwater's prairie-chicken. *Molecular Ecology, 25*, 4730-4744.

Bleu, J., Agostini, S., & Biard, C. (2017). Nest-box temperature affects clutch size, incubation initiation, and nestling health in great tits. *Behavioral Ecology, 28*, 793-802.

Burton, N.H.K. (2006). Nest orientation and hatching success in the Tree Pipit *Anthus trivialis*. *Journal of Avian Biology*, 37, 312–317.

Butler, M. W., Whitman, B. A., Dufty Jr., A. M. (2009). Nest box temperature and hatching success of American Kestrels varies with nest box orientation. *The Wilson Journal of Ornithology*, 121(4), 778-782.

Catry, I., Franco, A. M. A., Sutherland, W. J. (2011). Adapting conservation efforts to face climate change: Modifying nest-site provisioning for lesser kestrels. *Biological Consevation*, 144, 1111-1119.

Conrey, R. Y., Skagen, S. K., Adams, A. A. Y., & Panjabi, A. O. (2016). Extremes of heat, drought and precipitation depress reproductive performance in shortgrass prairie passerines. *International Journal of Avian Science*, 158, 614-629.

Cunningham, S. J., Martin, R. O., Hojem, C. L., & Hockey, P. A. R. (2013). Temperatures in excess of critical thresholds threaten nestling growth and survival in a rapidly-warming arid savanna: A study of common fiscals. *PLoS ONE*, 8.

Dawson, R. D., Lawrie, C. C., & O'Brien, E. L. (2005). The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. *Behavioral Ecology*, 144, 499-507.

Gaston, K. J., Blackburn, T. M., & Goldewijk, K. K. (2003). Habitat conversion and global avian biodiversity loss. *Proceedings of the Royal Society*, B (London) 270, 1293-1300.

Gleick, P. H., Adams, R. M., Amasino, R M., Anders, E., et al. (2010). Climate change and the integrity of science. *Science*, 328, 689-691.

Gowaty, P.A. & J. H. Plissner. 1998. Eastern bluebird (*Sialia sialis*). The birds of North America. Number 381.

Greño, J. L., Belda, E. J., & Barba, E. (2008). Influence of temperatures during the nestling period on post-fledging survival of great tit *Parus major* in a Mediterranean habitat. *Journal of Avian biology*, 39, 41-49.

Hurley, L. L., McDiarmid, C. S., Friesen, C. R., Griffith, S. C., & Rowe, M. (2018). Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences, 285.*

Kuitunen, M. & Alekonis, A. (1992). Nest predation and breeding success in common treecreepers nesting in boxes and natural cavities. *Ornis Fennica*, 69, 7-12.

Lambrechts, M. M., Wiebe, K. L., Sunde, P., Solonen, T., Sergio, F., Roulin, A., Møller, A. P., López, B. C., Fargallo, J. A., Exo, K.-M., Dell'Omo, G., Costantini, D., Charter, M., Butler, M. W., Bortolotti, G. R., Arlettaz, R., & Korpimäki, E. (2012). Nest box design for the study of diurnal raptors and owls is still an overlooked point in ecological, evolutionary and conservation studies: A review. *Journal of Ornithology*, 153, 23-34.

Larson, E. R., Eastwood, J. R., Buchanan, K. L., Bennett, A. T. D., & Berg, M. L. (2016). How does nest-box temperature affect nestling growth rate and breeding success in a parrot? *Emu*, 115, 247-255.

Larson, E. R., Eastwood, J. R., Buchanan, K. L., Bennett, A. T. D., & Berg, M. L. (2018). Nest box design for a changing climate: The value of improved insulation. *Ecological Management & Restoration*, 19, 39-48.

Libois, E., Gimenez, O., Oro, D., Mínguez, E., Pradel, R., & Sanz-Aguilar, A. (2012). Nest boxes: A successful management tool for the conservation of an endangered seabird. *Biological Conservation*, 155, 39-43.

Lloyd, J. D. & Martin, T. E. (2004). Nest-site preference and maternal effects on offspring growth. *Behavioral Ecology*, 5, 816-823.

Martínez-de la Puente, J., Merino, S., Lobato, E., Aguilar, J. R. D., del Cerro, S., Ruiz-de-Castañeda, R., & Moreno, J. (2010). Nest-climatic factors affect the abundance of biting flies and their effects on nestling condition. *Acta Oecologica, 36*, 543-547.

McDermott, M. E. & Degroote, L. W. (2016). Long-term climate impacts on breeding bird phenology in Pennsylvania, USA. *Global Change Biology*, 22, 3304-3319.

Meehl, G. A., Zwiers, F., Evans, J., Knutson, T., Mearns, L., and Whetton, P. (2000). Trends in extreme weather and climate events: issues related to modeling extremes in projections of future climate change. *Bulletin of the American Meteorological Society*, 81, 427–436.

Murphy, M. T. (1985). Nestling Eastern kingbird growth: effects of initial size and ambient temperature. *Ecology*, 66, 162-170.

Nilsson, S. G. (1984). The evolution of nest-site selection among hole-nesting birds: The importance of nest predation and competition. *Ornis Scandinavica, 15*(3), 167-175.

Pinowski, J., Haman, A., Jerzek, L., Pinowska, B., Barkowska, M., Grodzki, A., & Haman, K. (2006). The thermal properties of some nests of the Eurasian Tree Sparrow *Passer montanus*. *Journal of Thermal Biology*, 31, 573-581.

Purcell, K. L., Verner, J., Oring, L. W. (1997). A comparison of the breeding ecology of birds nesting in boxes and tree cavities. *The Auk*, 114, 646-656.

Reyna, K. S. & Burggren, W. W. (2017). Altered embryonic development in northern bobwhite quail (*Colinus virginianus*) induced by pre-incubation oscillatory thermal stresses mimicking global warming predictions. *PLoS ONE*, 12.

Robertson, R. J., & Rendell, W. B. (1990). A comparison of the breeding ecology of a secondary cavity nesting bird, the tree swallow (*Tachycineta bicolor*), in nest boxes and natural cavities. *Canadian Journal of Zoology, 68*, 1046-1052.

Rodríguez, S. & Emilio, B. (2016). Nestling growth is impaired by heat stress: an experimental study in a Mediterranean Great Tit population. *Zoological Studies*, 55.

Salaberria, C., Celis, P., López-Rull, I., & Gil, D. (2014). Effects of temperature and nest heat exposure on nestling growth, dehydration and survival in a Mediterranean hole-nesting passerine. *Ibis*, 156, 265-275.

Stanback, M. T., Mercandante, A. N., Cline, E. L., Burke, T. H., & Roth, J. E. (2013). Cavity depth, not experience, determines nest height in Eastern Bluebirds. *The Wilson Journal of Ornithology, 125*, 301-306.

Stanback, M. T., & Rockwell, E. K. (2003). Nest-site fidelity in Eastern Bluebirds (*Sialia sialis*) depends on the quality of alternate cavities. *The Auk, 120*, 1029-1032.

Tomás, G., Merino, S., Moreno, J., & Morales, J. (2007). Consequences of nest reuse for parasite burden and female health and condition in blue tits, *cyanistes* caeruleus. *Animal Behaviour, 73*, 805-814.

Vinoth, A., Thirunalasundari, T., Shanmugam, M., Uthrakumar, A., Suji, S., & Rajkumar, U. (2018). Evaluation of DNA methylation and mRNA expression of heat shock proteins in thermal manipulated chicken. *Cell Stress and Chaperones, 23*, 235-252.

Vedder, O., Zhang, H., & Bouwhuis, S. (2017). Early mortality saves energy: Estimating the energetic cost of excess offspring in a seabird. *Proceedings of the Royal Society B: Biological Sciences, 284*.

Wetzel, D. P. & Krupa, J. J. (2013). Where Are the Bluebirds of the Bluegrass? Eastern Bluebird Decline in Central Kentucky. *The American Midland Naturalist*, 169, 398-408.

Wiebe, K. L. (2001). Microclimate of tree cavity nests: is it important for reproductive success in Northern flickers? *The Auk*, 118, 412-421.