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EXPERIMENTALLY INCREASED INCUBATION TEMPERATURE AFFECTS  
POSTHATCHING DEVELOPMENT, OFFSPRING BEGGING AND PARENTAL  
CARE, AND NESTLING SURVIVAL IN THREE SPECIES OF WILD BIRD

by

Alexander J. Mueller

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Major: Biology

The University of Memphis

August 2018

## ACKNOWLEDGEMENTS

I would like to first acknowledge the support, encouragement, and influence of my family and loved ones, especially my parents Anna and George Mueller, without whom I could not have gotten to where I am today. I cannot begin to express my full gratitude to Dr. Keith Bowers who provided me the opportunity to join his lab and conduct research that is close to my heart at such a high level. He is an exceptional mentor, colleague, and friend that has been an ever-present source of support and guidance not only with this work but throughout all aspects of my graduate career. Drs. Freeman and Ferkin for being great committee members that have provided general advice and editorial assistance with this work. I would like to thank Kelly Danielle Miller for contributing a great deal to this research through hours of meticulous provisioning data collection, Jonathan Bradford Jenkins for assistance in establishing nestboxes and with fieldwork, and the Department of Biological Sciences at the University of Memphis for access to property for fieldwork. I also want to thank the Tennessee Ornithological Society and the UofM Department of Biological Sciences for financial support.

## **PREFACE**

The Bowers lab is broadly involved in several biological areas of study including, physiology, behavior, ecology, and evolution. However, all members are bound by the common thread of an inherent curiosity and appreciation of biology and are passionate about utilizing the scientific method to shed light on new and important aspects of the natural world. The following research was conducted to inform conservation efforts and to build upon the vast amount of work demonstrating the implications and importance of climate change. This co-authored manuscript has been formatted in the style of the journal to which it has been submitted for publication. All research activities were performed in accordance with the University of Memphis Institutional Animal Care and Use Committee, United States Geological Survey banding permit 24052, and Tennessee Wildlife Resources Agency Scientific Collection Permit 3950.

## ABSTRACT

It is now widely accepted that recent climatic changes have had a causal effect on changing avian life histories. However, evidence for this is largely observational, whereas cause-and-effect inference requires an experimental approach. Here, we assess effects of experimentally increased temperature during incubation on posthatching development in three species of wild songbird, the Carolina chickadee (*Poecile carolinensis*), Carolina wren (*Thryothorus ludovicianus*), and prothonotary warbler (*Protonotaria citrea*). Increased incubation temperatures (i) reduced the duration of the incubation and nestling periods, (ii) reduced posthatching begging for food by nestlings, and (iii) reduced posthatching survival in Carolina chickadees and prothonotary warblers, while nestling Carolina wrens had similar survival but reduced pre-fledging mass. Our results suggest that increasing environmental temperature affect fitness in wild populations in generally negative but species-specific ways, and induce life-history changes including the duration of development and the classic trade-off parents face between the size and number of offspring.

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## **CHAPTER 1**

**Experimentally increased incubation temperature affects  
posthatching development, offspring begging and parental  
care, and nestling survival in three species of wild bird**

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Keywords: carry-over effect, climate change, parental care, *Poecile carolinensis*,  
*Protonotaria citrea*, *Thryothorus ludovicianus*

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## **Introduction**

It is now commonly accepted that recent climatic change, including shifts in environmental temperature, impact the life histories of various organisms. These impacts include effects on the timing of migration and breeding phenology for various avian species (Both et al. 2004; Visser et al. 2009; Hurlbert & Liang 2012; Bowers et al. 2016b). However, few studies have focused on direct effects of temperature on parents and offspring during the breeding season outside of its effect on breeding phenology. One direct effect of temperate on avian life histories includes that on parental incubation effort

and embryonic development. Incubation of eggs is thought to be energetically costly (e.g., Reid et al. 2002; Hanssen et al. 2005; but see Sakaluk et al. 2018); however, because ambient temperatures are seldom high enough to foster embryonic development, it is a critical component of parental allocation (Ardia et al. 2009; Lothery et al. 2014; Berntsen and Bech 2016; Sakaluk et al. 2018). Perhaps not surprisingly, the extent of maternal effort dedicated to incubation is partly dependent on nest microclimate (Ardia et al. 2009). For example, Coe et al. (2015) found that female tree swallows (*Tachycineta bicolor*) vary in their incubation constancy and duration of incubation off-bouts, or the time spent off the nest, across a range of environmental temperatures. Off-bout frequency was also found to decrease as temperature increases (Coe et al. 2015). When incubating in a heated nest box, female birds have been found to maintain higher body condition, which might allow for enhanced incubation effort (Pérez et al. 2008).

In many taxa, the length of the incubation period is negatively correlated with temperature (Martin et al. 2007; Ardia et al. 2009, 2010; Nord and Nilsson 2011; Coe et al. 2015; Bowers et al. 2016*b*). This correlation may arise as a direct effect of ambient temperature on the rate of development, as a consequence of temperature-dependent parental incubation behavior, or a combination of both. With greater incubation constancy and decreased frequency of off-bouts, females may be able to maintain greater average temperatures of their eggs during the incubation period (Ardia et al. 2009; Coe et al. 2015). Increases in average egg temperatures during incubation not only accelerate development within the egg, but may have additional phenotypic effects that ultimately shape offspring survival and fitness (Berntsen and Bech 2016). For example, offspring hatching success was found to increase with warmer incubation temperatures (Nord and

Nilsson 2011). However, increases in temperature may not always be beneficial to avian offspring. Nest success has been found to decline under abnormally high daytime temperatures, particularly when nest microclimate exceeds an optimal temperature, e.g., 35°C (Ardia 2013), and an optimal incubation temperature (ca. 36°C) has been observed in wood ducks (*Aix sponsa*; DuRant et al. 2010, 2011, 2012). In other words, it appears that a strict temperature range must be maintained for maximal nest success.

Although increased temperatures during incubation can be beneficial under low average temperatures in early spring, the effects of temperature on the duration of incubation may generate costs in subsequent life-history stages. In an analysis of a 36-year data set from a study of a population of house wrens (*Troglodytes aedon*) in Illinois, Bowers et al. (2016b) found an advance in breeding phenology in the population associated with increasing temperatures since 1980, and that incubation periods had shortened over this time as well, as there was a negative correlation between ambient temperature and the duration of incubation (Bowers et al. 2016b). That study also revealed that nestling periods had lengthened during warmer breeding seasons, as incubation duration and the period of nestling growth were negatively correlated. Thus, optimal environmental temperature may differ for embryos and nestlings. However, it is important to note that these recent findings are observational by nature, and may be influenced by additional unexplored variables. For example, the increase in the length of the nestling stage since 1980 may be attributable to a direct effect of reduced incubation duration on the developmental state and posthatching growth of nestlings, but it could also be explained by changes in the quantity or quality of arthropod prey over this time that have coincided with increasing temperatures. Thus, while long-term observations are

essential, elucidating effects of environmental change ultimately requires an experimental approach.

Here, we use an experimental manipulation of environmental temperature during incubation to test the hypothesis that increasing incubation temperature affects both incubation duration and posthatching development within three species of woodland bird, the Carolina chickadee, Carolina wren, and prothonotary warbler. We heated experimental nests using battery-powered resistive heating coils, which successfully led to a subtle increase in environmental temperature of nests that is consistent with recent and forthcoming climatic changes. We predicted that increased temperature during incubation would reduce the time required for eggs to hatch, and that this would generate a potential cost through prolonging the period of nestling development. We also investigated effects of increasing incubation temperature on the development of avian young, pre- and post-hatching parental care, and consequences of these effects for parental reproductive success.

## **Methods**

### *Study species and field site*

Each of our three study species is distributed across the Southeastern United States. Each species is insectivorous and generally requires a preformed cavity in which to build a nest. Additionally, only the female incubates eggs and broods young nestlings, but both parents provision young with food after hatching (Petit 1999; Mostrum et al. 2002; Haggerty and Morton 2014).

Carolina chickadees are small (8-12 g), year-round residents of the Southeastern United States. In west Tennessee, egg production begins in mid-March and ceases by the end of May (Pitts 1998), and chickadees rarely produce second broods or replace failed first broods within a single season (Mostrum et al. 2002). Hatching begins 12-15 d after the start of incubation, and fledging occurs 16-19 d after hatching (Mostrum et al. 2002). Carolina Wrens (18-23 g) are also year-round residents of the Southeastern United States, with peak egg production spanning from April through most of July (Haggerty and Morton 2014). Incubation lasts 13-18 d (Gill and Haggerty 2012), and fledging occurs 10-16 d after hatching (Haggerty and Morton 2014). Unlike the chickadees and wrens, prothonotary warblers (14-16 g) are neotropical migrants, wintering in the West Indies, Central America, and northern South America (Stiles and Skutch 1989). Males arrive on their breeding grounds from late March through April across most of their range (populations in southern states settle earlier than those migrating farther northward); on average, females arrive several days after males (Petit 1999). The warblers breed in Tennessee from the end of April through July; ca. 50-75% of females will attempt a second brood after successfully fledging a first brood, with peak egg production occurring in May for the first brood and late June and early July for the second (Petit 1999). Incubation lasts 12-14 d, and fledging occurs 9-11 d posthatching (Petit 1999).

This study was conducted at the Edward J. Meeman Biological Station (35.363°N, 90.017°W) in west Tennessee, USA. We established nestboxes ( $N = 600$ ) for breeding birds before the 2017 field season within secondary deciduous and mixed forest habitat on the Loess Bluffs atop the Mississippi Alluvial Plain. Nestboxes for breeding chickadees ( $N = 380$ ; interior dimensions: 14.0 cm  $\times$  10.2 cm (length  $\times$  width) basal area,

with a pitched roof 22.9 cm high in the front and 25.4 cm high in the back of the nestbox) had an entrance hole 2.86 cm in diameter to allow access only to chickadees, and were distributed on a 45-m grid throughout ca. 75 ha of forest. Nestboxes for breeding Carolina wrens and Prothonotary warblers ( $N = 220$ ; interior dimensions: 14.0 cm  $\times$  10.2 cm (length  $\times$  width) basal area, with a pitched roof 14.0 cm high in the front and 15.4 cm high in the back of the nestbox) had a wider slot entrance (10.2 cm wide  $\times$  4.4 cm tall) beneath an eave of 3.8 cm, and were distributed on an 80-90 m grid over ca. 100 ha of forest.

### *Field Procedures*

Nestboxes were checked at least twice weekly for the formation of new nests. Within each species, the first nest was assigned a treatment by a coin flip, and treatments were alternated with each new nest thereafter. All nestboxes had a resistive film heater (i.e., heating coils) attached to the inside rear wall once the clutch was complete and incubation was underway (i.e., the first full day of incubation with a complete clutch). In both experimental and control nests, heaters were wired to a small battery pack outside the nest. Heaters were powered by four rechargeable AA batteries, which were replaced each morning during the period of the manipulation (each day of incubation). Control nests contained a heater and battery pack, and were visited by us with the same frequency as experimental nests, but the heaters were not turned on. All nests were equipped with two thermochron iButton dataloggers to record internal and external ambient temperature. Data loggers that measured internal temperature were located midway up the sidewall of the nestbox while external data loggers were placed underneath the

nestbox to obtain shaded ambient temperature. On the fourth day of incubation, we obtained digital video recordings of nests to document female incubation behavior (incubation constancy, number of on-/off-bouts). We used a digital video recorder on a 1.5-m pole ca. 1 m from the nestbox, which was placed the day before the recording so the birds would be habituated to its presence. We define incubation constancy as the proportion of time the female is within the nestbox during video observation, and each visit a female makes to the nest was marked as an on-/off-bout.

We captured adults at the nest either during the second half of incubation or while provisioning nestlings by either capturing them inside nestboxes or using mist nets placed outside the box. Upon capture, we weighed (.1 g) all adults using a digital scale, measured their tarsus length (.1 mm) with dial calipers, and the length (.5 mm) of their flattened wing cord and tail feathers using a stopped rule. All adults were also banded with a unique U.S. Geological Survey (USGS) aluminum leg band. Both female and male chickadees and wrens, and male warblers, were also banded with 3 additional colored leg bands arranged in unique combinations so they could be visually identified and observed without capture.

Once eggs hatched, we subsequently monitored nests until fledging. Four days after hatching, which corresponds to the period of most-rapid growth in each of our three study species, we observed parental provisioning using digital videos as described above for incubation behavior. Observations lasted at least 1 hr, which provides a representative sample of consistent individual differences in parental behavior over longer time spans and multiple days (Murphy et al. 2015). During these observations we recorded food deliveries by the male and female parents, fecal sacs removed by parents as a measure of

nest sanitation, and, for females, the amount of time she spent in the nest brooding her young, which can often have a positive effect on nestling growth and size (Bowers et al. 2015). At this time, we also recorded nestling begging vocalizations using a microphone inside the nest with a digital voice recorder outside the box (following Barnett et al. 2011; Bowers et al. 2016a), and we used Raven Pro 1.5 sound analysis software (Cornell Lab of Ornithology) to count the begging vocalizations (Bowers et al. 2016a). All nestlings were processed prior to fledging, during which time they were weighed (.1 g) and had their tarsus length measured (.1 mm) using dial calipers to obtain a measure of skeletal size. Nestlings were banded with a unique USGS leg band for individual identification, and weighed for the last time at different ages for the three species to reflect pre-fledging mass; Carolina chickadees were banded at 12 days, Carolina wrens at 9 days, and prothonotary warblers at 8 days posthatching (where hatching date is the day on which hatching begins for a given nest). Nestlings also had a small blood sample drawn through brachial venipuncture to determine hematocrit (percent of blood comprised of red blood cells), a measure of overall health state (Bowers et al. 2014). Neonatal body mass prior to independence is often a strong predictor of the subsequent recruitment of offspring as breeding adults into local populations in a variety of species (e.g., Clutton-Brock et al. 1987, Tinbergen and Boerlijst 1990; Both et al. 1999; Bowers et al. 2014), and hematocrit is also predictive of recruitment, but the relationship between hematocrit and recruitment is hump-shaped (Bowers et al. 2014). We subsequently visited nests daily after banding to check for fledging. The total number of nests within each treatment was evenly represented within each species, and equally represented overall (table 1).

### *Statistical Analyses*

All analyses were conducted using SAS (ver. 9.4), with two-tailed hypotheses ( $\alpha = .05$ ). We first analyzed the temperature of nest microclimates using both the raw internal temperature of a nestbox and the difference between internal temperature and shaded ambient temperature outside the nestbox. We assessed these using nested ANOVAs with treatment as a fixed effect and nest within treatment as a random effect to account for the non-independence of observations within the same nest. We tested for an effect of our treatment on maternal incubation behavior at the time of day during which our heating coils were active, assessing this using a multivariate ANOVA (MANOVA) with incubation constancy and the number of on/off bouts per hour as dependent variables, with treatment and species as crossed fixed effects, and we also included clutch-initiation date as a covariate to control for environmental variation. We then tested for an effect of the manipulation on hatching success (i.e., the proportion of eggs laid that hatched) using a linear mixed model with treatment and species as crossed main effects and clutch size as a covariate.

We then assessed effects of our treatment on the duration of the incubation and nestling-rearing stages using a MANOVA with these two dependent variables, and included treatment and species as crossed main effects with clutch-initiation date as a covariate. We analyzed nestling begging vocalizations on day four posthatching using a linear model with treatment and species as crossed main effects, and hatching date and brood size as covariates. We then included these nestling begging vocalizations as an independent variable in a MANOVA assessing parental care. To assess parental care, we used principal components analysis on maternal provisioning, brooding time, and fecal

sac removal, reducing the number of dependent variables to two factors that we retained, which explained 74% of the variation in maternal care. The first loaded strongly and positively on maternal provisioning rate ( $r_{47} = .777$ ,  $P < .0001$ ), and to a slightly lesser extent on fecal sac removal ( $r_{47} = .767$ ,  $P < .0001$ ), and the second loaded strongly and positively on maternal brooding time ( $r_{47} = .971$ ,  $P < .0001$ ). For males, we initially included two variables, provisioning rate and fecal sac removal, and retained one factor that explained 70% of the variation in male parental care and which loaded strongly and positively on both provisioning rate ( $r_{47} = .834$ ,  $P < .0001$ ) and fecal sac removal ( $r_{47} = .834$ ,  $P < .0001$ ). We then used these three factors describing parental care within pairs (female provisioning and fecal sac removal, female brooding time, and male provisioning and fecal sac removal) as dependent variables in a MANOVA with treatment and species as crossed main effects, and offspring begging frequency, hatching date, and brood size as covariates. We then used a linear model to test for an effect of temperature treatment on the number of young surviving to fledge from the nest, including treatment and species as crossed main effects with clutch size as a covariate to reflect the number of young fledged per egg laid (e.g., Hodges et al. 2015). Finally, we analyzed nestling pre-fledging body mass using a linear mixed model with nest as a random effect to account for the non-independence of nestlings within broods, and we included in this model treatment and species as crossed main effects with the day of the year on which nestlings were banded and weighed as a covariate; we also included tarsus length as a covariate to control for skeletal size, such that our analysis is reflective of body condition or size-adjusted body mass (e.g., García-Berthou 2001; but see also Barnett et al. 2015).

## Results

### *Effects of heating treatment on environmental temperature within nests*

Experimental nests were significantly warmer, on average, than control nests from 7:00–13:00 (i.e., the time during which our heating coils were active) (mean  $\pm$  SE; experimental:  $24.6 \pm .4$  °C, control:  $23.2 \pm .4$  °C;  $F_{1,49} = 7.55$ ,  $P = .0084$ ; (fig. A1, available online). Temperature differentials between internal and external iButton measures confirmed that the temperature of experimental nest microclimates was elevated above ambient to a greater extent than for control nests, particularly while the heating coils were active ( $F_{1,49} = 24.84$ ,  $P < .0001$ ; fig. 2A), and temperature over the entire day was marginally but non-significantly elevated over the entire day in experimental nests ( $F_{1,49} = 3.88$ ,  $P = .0545$ ; fig. 2B). Thus, the manipulation resulted in a subtle, yet noticeable, increase in temperature of the nest as a whole.

We assessed maternal incubation constancy using direct observations during the time that heating coils were active. Despite the increase in temperature within experimental nests, there was no effect of treatment on maternal incubation constancy (table 2). Further, the proportion of eggs laid that subsequently hatched was not affected by our treatment ( $F_{1,42} = .03$ ,  $P = .8738$ ) nor by a treatment  $\times$  species interaction ( $F_{2,42} = 1.34$ ,  $P = .2729$ ).

### *Duration of incubation and the nestling stage*

Overall, the duration of both the incubation and nestling stages was reduced by the increase in incubation temperature (table 3; fig. 2). Although there was no interaction between treatment and species in their effects on these variables overall (table 3), the

length of the nestling stage was reduced specifically for Carolina wrens in experimental nests relative to controls. This overall treatment effect was driven by changes in both incubation and nestling stages (see standardized canonical coefficients, table 3); that these coefficients are of the same sign is indicative of a positive correlation overall between incubation duration and the length of the nestling stage.

#### *Posthatching development and parental care*

The experimental increase in incubation temperature carried over to affect nestling development after hatching. Nestlings that developed under increased incubation temperatures solicited food from their parents at a lower rate than control nestlings (fig. 3A; treatment:  $F_{1, 40} = 5.96, P = .0191$ ; species:  $F_{2, 40} = 6.90, P = .0027$ ; treatment  $\times$  species:  $F_{2, 40} = .60, P = .5528$ ), while controlling for variation in hatching date ( $F_{1, 40} = .00, P = .9919$ ) and brood size ( $F_{1, 40} = .54, P = .4684$ ). Nestling begging vocalizations did not directly predict patterns of parental care, but there was a significant interaction between treatment and species in their effect on parental care (table 4). Follow-up tests to tease apart this interaction revealed that, within the Carolina wrens, experimental females spent significantly less time brooding their young after hatching than control females (effect of treatment within Carolina wrens:  $F_{1, 39} = 5.07; P = .0301$ ; fig. 3B). No other differences within species in maternal or paternal care were significant (all  $P > .05$ ). Of the variables we analyzed describing maternal and paternal care, maternal brooding time had the greatest effect on nestling size-adjusted body mass prior to fledging (estimate  $\pm$  SE =  $.367 \pm .161, F_{1, 42} = 5.17, P = .0281$ ; fig. 3C), but neither maternal nor paternal provisioning had any effect on pre-fledging condition (both  $P > .2$ ).

### *Direct effects of incubation temperature on nestling survival and body condition*

Although there was no difference in clutch size ( $F_{1, 45} = .01, P = .9217$ ) or hatching success (above) between control and experimental nests, experimental nests experienced increased posthatching mortality overall, such that experimental nests produced fewer fledglings per egg laid than control nests (table 4; fig. 4A). While controlling for date (table 4; parameter estimate  $\pm$  SE =  $-.025 \pm .011$ ), we also detected an interaction between treatment and species in their effect on nestling condition (table 4; fig. 4B); follow-up tests revealed that Carolina wren nestlings in experimental nests were in poorer condition, on average, than were control nestlings ( $F_{1, 36.5} = 4.29, P = .0456$ ; fig. 4B), while there was no effect of incubation treatment on pre-fledging mass in either prothonotary warblers ( $F_{1, 37} = 2.01, P = .1651$ ) or Carolina chickadees ( $F_{1, 32} = .67, P = .4203$ ).

### **Discussion**

To date, a variety of methods has been used to alter the nest microclimate experimentally. Our approach caused an increase in nestbox temperatures that effectively extended peak daytime temperatures into the morning hours. Comparisons of raw internal temperatures of nests and temperature differentials (within nest relative to ambient temperature outside the nest) reveal a clear distinction in temperatures between control and experimental nests. Moreover, our manipulation produced ecologically relevant temperatures consistent with recent and forthcoming climatic changes. On average, temperature differentials did not exceed 2°C for experimental nests during the time that our heaters were active. We observed no effect on hatching success between groups, which might

have been indicative of “overheating” eggs. However, the subtle, yet statistically significant, increase in temperature during incubation led to changes in nestling development and post hatching survival. We also did not observe any difference in maternal incubation behavior between treatments, suggesting that the effects we observed are attributable to differences in incubation temperature *per se*.

### *Incubation and Nestling Duration*

Not unexpectedly, we found that increasing incubation temperature shortens the duration of embryonic development overall, consistent with previous results from both observational and experimental studies (e.g., Martin et al. 2007; Ardia et al. 2009, 2010; Nord and Nilsson 2011; Coe et al. 2015; Bowers et al. 2016*b*). We also predicted that increases in incubation temperature would influence the duration of the nestling stage. In a recent observational study, while the increase in environmental temperature that had occurred over recent decades led to a shortening of the incubation period, the length of the nestling stage had grown, and the duration of the incubation and nestling stages were negatively correlated (Bowers et al. 2016*b*). This may not be without costs in natural populations if the prolonged time required for nestling development leads to increased probability of nest depredation or parasitism (Remeš and Martin 2002; Roff et al. 2005; Remeš 2006, 2007; Chiavacci et al. 2015), or reproductive costs to parents associated with extended periods of postnatal care. We predicted that this negative correlation would also be reflective of constraints on nestling development induced by the rate of embryonic development (i.e., shorter incubation times necessitate longer nestling periods). This was not the case, however, in our experimental study, which revealed a

positive correlation between these variables. Thus, the negative correlation detected by Bowers et al. (2016b) over several decades might not be a direct effect of incubation duration on nestling development, but an indirect effect mediated by changes in food availability over time that lead to longer periods of posthatching development before nestlings are able to fledge (Carrier and Auriemma 1992; Michaud and Leonard 2000; Day and Rowe 2002; Bowers et al. 2013).

A reduction in the duration of these early life-history stages may have a number of consequences for the fitness of parents and offspring. Advances in fledging age could potentially benefit parental survival by reducing energy allocated to provisioning and allowing a greater investment into self-maintenance, but this can often have detrimental effects on offspring. Typically, offspring that fledge from the nest earlier within the breeding season have significantly increased recruitment as breeding adults in local populations relative to nestlings produced later in the breeding season (van Noordwijk et al. 1995; McCleery et al. 2004; Williams 2012; Bowers et al. 2013, 2014, 2016; Visser et al. 2015). The higher abundance of arthropod prey, along with more time to mature before autumn migration or winter, should, therefore, provide earlier-fledged offspring advantages over those that fledge later in the season. Although fledging at an earlier age and premature developmental stage reduces recruitment generally (e.g., Bowers et al. 2013), nestlings produced later within breeding seasons may benefit from earlier fledging if this relaxes the extent of resource competition among siblings within nests.

### *Nestling Survival*

One major aim of our study was to test for effects of increasing incubation temperature that carry over to influence posthatching survival, and, thus, parental fitness. Here, we detected species-specific responses to increased environmental temperatures, and these responses may have consequences at the population level, including the ability to adapt to climate change, as the number of young fledged within a year is among the strongest determinants of fitness in animals (e.g., McCleery et al. 2004). Specifically, there was no effect of incubation treatment on the number of young fledged by Carolina wrens, yet offspring pre-fledging body condition, an indication of phenotypic “quality” (Bowers et al. 2014), was reduced within experimental nests relative to controls. Conversely, our manipulation also affected Carolina chickadees and prothonotary warblers, as offspring quality in experimental nests was maintained at the same level as for control offspring, but with an effect on posthatching mortality such that experimental pairs fledged a lower number of young overall. Thus, we see between-species divergence in life-history responses to increasing temperature, including effects on that classic trade-off parents face between the size and number of offspring (Smith and Fretwell 1974).

The species-specific effects we observed on nestling survival may also have consequences at the community level. Investigating trends reported by the Breeding Bird Survey (Sauer et al. 2017) reveals statistically significant, range-wide decreases in the abundance of both prothonotary warblers and Carolina chickadees (i.e., the two species for which we observed a reduction in posthatching survival under increasing incubation temperature) since the onset of the survey in 1966. Conversely, the Carolina wren, the species for which we did not observe a reduction in posthatching survival, has

significantly increased in abundance throughout their range over this time (Sauer et al. 2017). These three species occupy a similar ecological niche, and it is likely that they experience interspecific competition over nesting sites, as each species requires a cavity in which to build their nest (*sensu* Slagsvold 1978; Newton 1994*a,b*). Our study, thus, provides a potential explanation for the patterns in abundance observed across these species' ranges, and suggests that increasing global temperatures are to blame, at least in part, for continuing declines in prothonotary warblers and Carolina chickadees.

Although increased mass often predicts the survival of avian offspring, increasing global temperatures may provide an exception to this rule. With gradually increasing temperatures, some studies indicate that reductions in body mass might actually be beneficial (Gardner et al. 2011; Andrew et al. 2018). Presumably, a smaller body dissipates heat more rapidly than a larger body because of the increased ratio of body surface area to volume. Thus, even if being in poorer condition tends to reduce survival, all else being equal, warmer environments may favor individuals who can more easily dissipate heat. For the Carolina wren, lighter offspring may be better suited to warmer environments, and a reduction in maternal brooding time may indicate that warmer temperatures allow females to invest relatively more energy toward self-maintenance. Whether this hypothesis is true, and whether such a process actually causes the growth observed in Carolina wren abundance, awaits further study.

### *Caveats*

It must be acknowledged that the patterns we have observed in this study are derived from a single year, the initial year in which these breeding populations were established

at our field site. As such, we advise caution in generalizing our results to other contexts. Nonetheless, the environmental conditions experienced during the 2017 breeding season were not particularly anomalous, and so we consider it unlikely that the patterns we report are a response to unusual environmental conditions experienced during the breeding season. Moreover, while we obtained a sample of nests that yielded adequate statistical power for detecting the effects we report, we acknowledge that our sample of Carolina chickadees is not large, as this species requires many years to approach a plateau in nestbox occupancy (Pitts 1998). However, given the consistency in the effects observed on the chickadees with those of the other species, particularly the warblers, it seems unlikely that our data are indicative of type I error.

### *Conclusions*

Both incubation duration and the nestling period were shortened under increased incubation temperature, and the potential consequences of these reductions in the duration of these life stages for parents and offspring are many and varied. In addition to changes in the timing of developmental stages, we detected direct effects on offspring phenotype, including their begging behavior, and on posthatching survival. The range of phenotypic plasticity in response to environmental temperatures may not be without limits, and, thus, may ultimately determine which species can successfully cope with rapid environmental change. The effects of rising temperatures, including those on nestling development, are likely already acting on avian populations. Future work will shed light on how changing temperatures affect the various stages of the life histories of these species, including their ability, or inability, to respond to change.

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**Table 1:** Nest occupancy for the 2017 field season.

Species	Number of active nests	Control	Experimental
Carolina chickadee	4	2	2
Carolina wren	17	9	8
Prothonotary warbler	31	15	16
Total	52	26	26

**Table 2:** Summary of effects on incubation constancy and the number of on/off bouts.

Significant effects in bold type.

Effect	<i>Pillai's</i> <i>Trace (F)</i>	df	<i>P</i>	Standardized Canonical Coefficients	
				Incubation constancy	On/off bouts
Treatment	.05	2, 41	.9493	.721	1.641
Species	<b>5.79</b>	<b>4, 84</b>	<b>.0004</b>	<b>.021</b>	<b>-1.188</b>
Clutch-initiation date	<b>4.97</b>	<b>2, 41</b>	<b>.0116</b>	<b>1.502</b>	<b>1.564</b>
Treatment × species	.43	4, 84	.7954	.812	-.394

**Table 3:** Summary of effects on incubation duration and the length of the nestling stage. Significant effects in bold type.

Effect	<i>Pillai's</i> <i>Trace (F)</i>	df	<i>P</i>	Standardized Canonical	
				Coefficients	
				Incubation	Nestling stage
Treatment	<b>5.69</b>	<b>2, 37</b>	<b>.0070</b>	<b>1.517</b>	<b>.604</b>
Species	<b>41.74</b>	<b>4, 76</b>	<b>&lt; .0001</b>	<b>.418</b>	<b>2.788</b>
Clutch-initiation date	<b>5.54</b>	<b>2, 37</b>	<b>.0079</b>	<b>1.400</b>	<b>-1.334</b>
Treatment × species	1.92	4, 76	.1151	1.525	.538

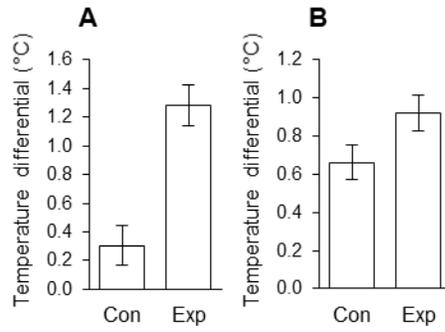
**Table 4:** Summary of effects on posthatching parental care. Significant effects in bold type.

Effect	<i>Pillai's</i>		Standardized Canonical Coefficients			
	<i>Trace (F)</i>	df	<i>P</i>	Maternal food deliveries	Maternal brooding time	Male food delivery and nest sanitation
Treatment	.87	2, 37	.4673	.14	-.972	.8
Species	<b>3.85</b>	<b>6, 76</b>	<b>.0021</b>	<b>1.087</b>	<b>.623</b>	<b>.138</b>
Brood size	<b>5.73</b>	<b>3, 37</b>	<b>.0025</b>	<b>.047</b>	<b>1.328</b>	<b>-.340</b>
Hatching date	2.8	3, 37	.0535	1.084	.119	-.135
Begging frequency	.46	3, 37	.7148	.818	-.238	.877
Treatment × species	<b>2.45</b>	<b>6, 76</b>	<b>.0348</b>	<b>.107</b>	<b>1.208</b>	<b>-.558</b>

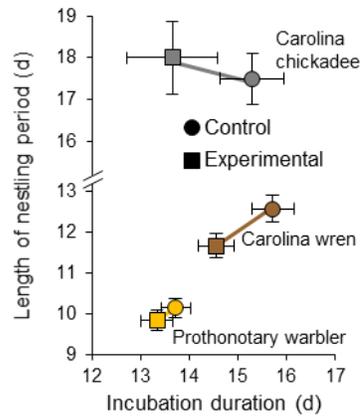
Note: Maternal food deliveries and brood time are principal components loading strongly on each of those components of maternal care, whereas the one major axis in male care loaded strongly on both food delivery and nest sanitation (i.e., fecal sac removal), which were strongly positively correlated.

**Table 5:** Effects on the number of young fledged and pre-fledging body mass of nestlings. Significant effects in bold type.

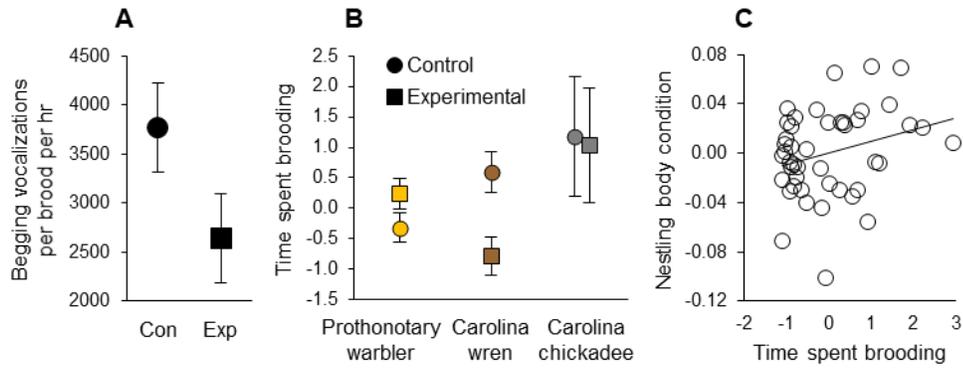
Effect	<i>F</i>	df	<i>P</i>
Number of young fledged			
Treatment	<b>4.50</b>	<b>1, 41</b>	<b>.0400</b>
Species	.82	2, 41	.4469
Clutch size	<b>5.37</b>	<b>1, 41</b>	<b>.0255</b>
Treatment × species	1.82	2, 41	.1748
Pre-fledging body mass			
Treatment	.13	1, 33.1	.7210
Tarsus length	<b>174.49</b>	<b>1, 149</b>	<b>&lt; .0001</b>
Species	<b>11.2</b>	<b>2, 49.3</b>	<b>&lt; .0001</b>
Banding date	<b>5.33</b>	<b>1, 37.1</b>	<b>.0267</b>
Treatment × species	<b>3.49</b>	<b>2, 34.3</b>	<b>.0419</b>



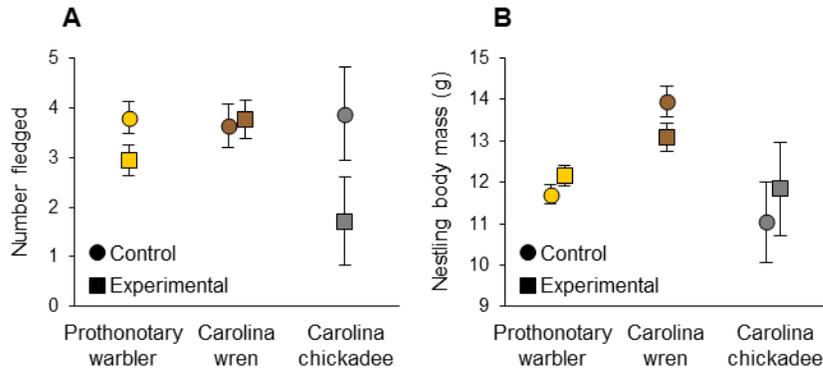
**Figure 1:** Temperature differentials of internal and external temperature measurements for each treatment. (A) Cropped data set only comparing temperatures between 7:00-13:00, (B) Full data set comparing all temperature records during incubation. Plotted are least-squares means  $\pm$  SE.



**Figure 2:** Incubation and nestling duration for all three species measured in days. Plotted are least-squares means  $\pm$  SE.



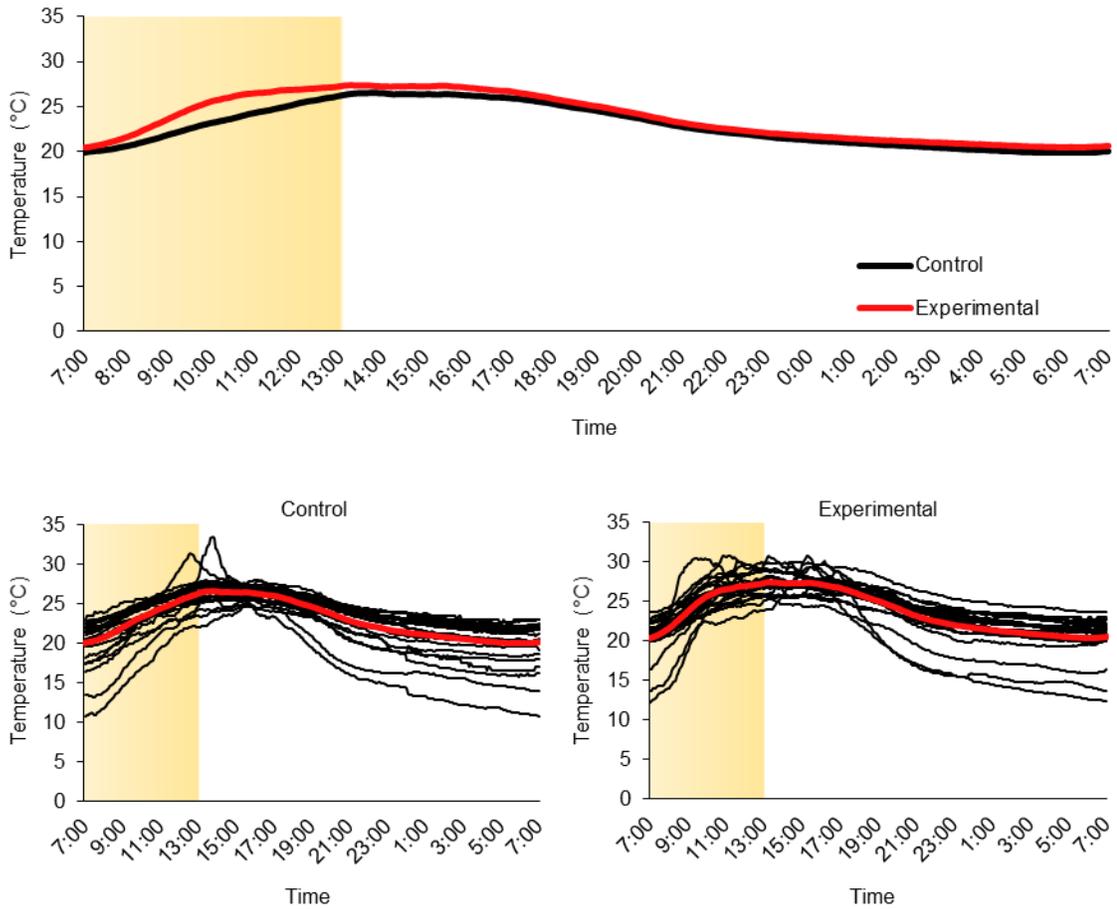
**Figure 3:** (A) Nestling begging vocalizations four days post-hatching, observed during our observations of parental care. (B) Species-specific effects on the amount of time females spent brooding their young, derived from a principal component of maternal behavior that loaded strongly on brooding time. (C) Effect of maternal brooding time on nestling pre-fledging body condition across the three species. Body condition here is calculated as the residual of a  $\log_{10}(\text{mass})$   $\log_{10}(\text{tarsus})$  linear regression for graphical purposes.



**Figure 4:** Nestling survival (A) and pre-fledging body mass (B) by species and treatment.

Plotted are least-squares means  $\pm$  SE.

## Appendices



**Figure A1:** Internal iButton temperature measurements. The top panel compares overall treatment means for all nests on each day of incubation averaged over 24 h. In the lower two panels, black lines represent reaction norms for individual nests, calculated as the mean from each day of incubation within a given nest; red lines represent mean temperature values for that treatment overall. The shaded area depicts the time when most heaters would have been active for experimental nests.