THE EFFECTS OF VARIATION IN TEMPERATURE AND PARENTAL BEHAVIOR ON OFFSPRING BODY MASS, TELOMERES AND SURVIVAL ARE CONTEXT-DEPENDENT IN FREE LIVING HOUSE SPARROWS (*PASSER DOMESTICUS*)

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MASTER OF SCIENCE

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ABSTRACT

Although developing birds are vulnerable to extreme and erratic temperature conditions associated with climate change, parents have some ability to buffer these effects via incubation and postnatal behavior. However, parents are constrained by their own physiology and ecology. In this thesis, I sought to determine which factors (seasonal thermal profile, consistency of ambient temperature and/or parental behavior) drove traits linked to fitness across ontogeny in free-living house sparrow nestlings (*Passer domesticus*). I found that the effects of these factors were context-dependent; seasonal thermal profile and average temperature were important in shaping body size across ontogeny, but variance in nest temperature and female postnatal visits better predicted hatching and day 10 survival, respectively. Future studies should seek to answer these questions in other populations and explore hypotheses surrounding interactions between developmental environments to better our understanding of climate change and thermoregulation in response to increasingly warm and erratic global temperatures.

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CHAPTER 1. INTRODUCTION

As the effects of climate change progress, organisms in terrestrial environments are likely to be impacted by increasingly warm and erratic conditions (DuRant et al., 2019; Thornton et al., 2014). Ectothermic organisms, those that gain the majority of their body heat from their environment (Tzschentke & Rumpf, 2011; McCue, 2004), are likely to be especially vulnerable to these effects, and the costs incurred by extreme conditions have already become evident organisms inhabiting the tropics and subtropics (Clusella-Trullas & Chown, 2014). Adult birds are endothermic, in that they gain the majority of their body heat from metabolic processes (McCue, 2004, Tzschentke & Rumpf, 2011), but their developing young transition from ectothermy to endothermy during development. In altricial birds (those hatching at an early stage of development; Augustine et al., 2018), this transition does not typically occur until around five days post-hatching (Andrew et al., 2017). Therefore, during embryonic development and early postnatal growth (the period from after hatching until when they leave the nest to fledge), birds are still highly vulnerable to their environmental conditions.

In developing birds, both thermal extremes and variability can influence growth outcomes, survival and longevity. Exposure to very cold temperatures can slow developmental rate (Nord & Nilsson, 2021; Japanese quail; Vedder et al., 2018; common tern) and increase telomere length (Vedder et al., 2018). Telomeres are protective complexes found at the end of eukaryotic chromosomes, and they are predictive of individual variation in lifespan (Vedder et al., 2018; Stier et al., 2020). Conversely, exposure to very warm temperatures can increase developmental rate (Stier et al., 2020; Japanese quail; Ospina et al., 2018; American robin), decrease hatching success (Wada et al., 2015; zebra finch, Carvalho et al., 2020; Japanese quail) and shorten telomeres (Stier et al., 2020). Exposure to highly variable conditions (i.e., artificial incubation recesses or egg

cooling via removal from the nest) can slow developmental rate during incubation (Stier et al., 2020) and postnatal growth (Rubin et al., 2021; zebra finch) and can decrease telomere length (Stier et al. 2020).

Birds select microclimates (Carroll et al., 2018), build nests (Heenan, 2013), and also take part in incubating and brooding in order to prevent egg and nestling cooling (Yoon et al., 2016), protect eggs from extreme temperatures, and maintain a consistent environment (Clauser & McRae, 2017). In addition to influencing the temperatures experienced by embryos and early nestlings (Heenan, 2013), ambient temperature can influence parental incubation and brooding behavior. As temperatures warm, female yellow warblers typically spend less time on the nest during incubation (Rowher & Purcell, 2019), and species such as Cape rockjumpers spend less time brooding in the presence of high temperatures (Oswald et al., 2021). Ambient temperature can also impact parental feeding behavior, which can indirectly impact nestling growth and survival outcomes. In Cape rockjumpers, nestling feeding rate can decrease with ambient temperature (Oswald et al., 2021), and prey availability of insectivores such as tree swallows shifts with ambient temperature, leaving nestlings highly vulnerable to sudden cold snaps (Shipley et al., 2020).

However, despite evidence for plasticity in parental behavior, parents are constrained by their physiology and ecology. Incubating birds can have a 20-50% higher metabolic rate than non-incubating birds (DuRant et al., 2013), and further, incubation in the presence of thermal extremes can be especially costly for parents (Vleck, 1981). Parents taking part in any form of parental care also lose out on valuable time to forage for themselves and take part in other forms of self-maintenance (Álvarez & Barba, 2014). Parents continually must choose between prioritizing the care of their offspring and their own self-maintenance and future reproductive potential, and as

temperatures associated with climate change become more extreme and variable, it is unclear what parents will decide to prioritize (DuRant et al., 2019; Thornton et al., 2014).

Historically scientists have largely ignored variance as an explanatory variable (Williams, 2008). This is problematic because climate change induces an increase in thermal variability (Thornton et al., 2014) as well as in averages of ambient conditions, and with unpredictable spikes and drops in temperature, parents may struggle to maintain consistent microclimate thermal conditions via incubation and brooding (DuRant et al., 2019; Clauser & McRae, 2017; Oswald et al., 2021). Food availability could also be influenced by erratic ambient temperatures, as temperature dictates insect flight patterns and therefore prey abundance for nestlings (Shipley et al., 2020). Therefore, it is imperative to determine what drives developmental outcomes in free-living birds, whether it is thermal extremes, consistency or parental behavior across ontogeny, as this knowledge could be applied to protect avian species especially vulnerable to the effects of climate change. Currently, how each of these factors shape early- and late-stage nestling fitness traits is unclear.

In the second chapter of this thesis, I first assess the relative importance of seasonal thermal profile (a principle component encompassing averages and extremes in ambient and nest temperature as well as date) and variance in ambient temperature and nest microclimate in shaping early and late growth, telomere and survival outcomes in house sparrow (*Passer domesticus*) nestlings. In the third chapter, I seek to clarify what drives these same early and late developmental outcomes by examining the contributions of ambient temperature and parental behavior across incubation and postnatal developmental periods. I hypothesize that developing embryos are vulnerable to inconsistent and extreme conditions, so exposure to these conditions will negatively impact developmental outcomes. Therefore, parents modify behavior in response to ambient

temperature in order to modulate developmental environment and maximize fitness outcomes, and parents who successfully maintain tight regulation of the developmental environment through increased attentiveness will have the best nestling outcomes.

CHAPTER 2. SEASONAL THERMAL PROFILE AND VARIANCE IN TEMPERATURE DRIVE DEVELOPMENTAL OUTCOMES IN THE HOUSE SPARROW (*PASSER DOMESTICUS*) IN A CONTEXT-DEPENDENT MANNER

Introduction

Shifts in temperature extremes associated with climate change are expected to be highest in terrestrial environments (reviewed in DuRant et al., 2019), and in addition to ambient temperature, weather variability is expected to increase (Thornton et al., 2014). While the metabolism of terrestrial endotherms such as birds is less dictated by the environment alone, these organisms can still suffer costs related to extreme ambient temperatures. Endothermic organisms gain most of their body heat from metabolic processes, while ectothermic organisms gain the majority of their body heat from the environment (McCue, 2004; Tzschentke & Rumpf, 2011). Altricial birds (those hatching at an early stage of development; Augustine et al. 2018) transition from ectothermy to endothermy approximately five days post-hatching (Andrew et al., 2017), meaning that during this point in development, birds are essentially terrestrial ectotherms. Developing young are generally more impacted by their environmental temperatures than adults are because they are less able to buffer these effects.

Exposure to extremes in temperature and thermal variation during early development can influence growth trajectories, survival and longevity in a myriad of ways. Rates of embryonic development generally slow in the presence of cold (Nord & Nilsson, 2021; Japanese quail; Vedder et al., 2018; common tern) and highly variable (Stier et al., 2020; Japanese quail) incubation conditions, and increase in the presence of high temperatures (Stier et al., 2020; Ospina et al., 2018; American robin). Effects of unstable temperature conditions during incubation can persist and slow postnatal growth rates (Rubin et al., 2021; zebra finch). Survival decreases with exposure

to low (Berntsen & Bech, 2016; zebra finch) and high (Wada et al., 2015; zebra finch; Stier et al., 2020; Carvalho et al., 2020; Japanese quail) incubation temperatures. Warm (Vedder et al., 2018, Stier et al., 2020) and unstable (Stier et al., 2020) incubation temperatures can also shorten telomere length, with effects persisting into adulthood (Stier et al., 2020).

Telomeres are DNA-protein structures located at the ends of chromosomes to maintain stability, and they predict individual variation in lifespan across species (Vedder et al., 2018). Telomeres shorten with number of cell divisions (and therefore, age; Vedder et al., 2018) because of the "end-replication problem" and oxidative stress that induces DNA damage (Stier et al., 2020). The "end-replication problem" exists because during DNA replication, DNA polymerase cannot fully replicate the linear ends of DNA (Levy et al., 1992), and oxidative stress results from the production of reactive-oxygen species (ROS) during cellular respiration (Yubero-Serrano et al., 2014). Mechanisms such as the Alternative Lengthening of Telomeres (ALT) pathway and expression of telomerase exist to restore and elongate telomeres, but generally their activity decreases in adult somatic tissues (Stier et al., 2020).

The magnitude and variability of conditions an avian embryo experiences during development that could impact telomeres and other outcomes is driven by a combination of abiotic factors and parental behavior. Nest microclimate is shaped by ambient temperature, humidity, wind, solar radiation and gas composition. One reason birds build nests is to insulate their eggs, and in many species, parents change how they construct their nests with weather and date (Heenan, 2013). Before constructing a nest, birds may chose favorable nest sites to further decrease the likelihood of exposure of their offspring to extreme temperatures. During incubation, parents then create a consistent thermal environment for their developing young via incubation behavior, and can modify their attentiveness with ambient conditions (Carroll et al., 2018). For instance, avian

parents in colder climates often take shorter off-bouts to avoid egg cooling (Conway & Martin, 2000; orange-crowned warbler). However, there are limits to how well parents can buffer their young against ambient conditions, as incubation, especially rewarming cooled eggs, is costly to parents in general (Vleck, 1981). While avian parents help create a highly consistent thermal developmental environment in comparison to other terrestrial oviparous organisms (Vleck & Hoyt, 1991), parents are constrained by their physiology (Carroll et al., 2018) and the need for time for their own self-maintenance (Álvarez & Barba, 2014). Therefore, developing embryos will unavoidably experience some fluctuations in their thermal environment during development.

Historically, it has been extremely rare for authors to formally analyze inter-individual variation and variances in their data (Williams, 2008). This approach is problematic in that organisms in the field experience a great deal of environmental variation in their lives (Greives & Bowden, 2019), and this variation may influence outcomes of interest. Further, as climate change progresses, temperature conditions are expected to be both higher and more variable (Thornton et al., 2014), so it is crucial to thoroughly characterize the effects of temperature variability on developmental outcomes in order to inform conservation decisions for vulnerable avian species.

To determine the degree to which variance and seasonal thermal profile (a principal component encompassing extremes, averages and date) associated with nest microclimate and ambient temperature can influence avian developmental outcomes, iButton temperature loggers were placed in house sparrow nests experiencing naturally varying incubation conditions. I was then able to associate those conditions with nestling survival, body size and telomere length across development. I hypothesized that developing embryos are vulnerable to inconsistent and extreme temperatures, so exposure to these conditions will negatively impact developmental outcomes. I

predicted that embryos developing under inconsistent ambient and (especially) nest temperatures will have lower hatching success and shorter telomeres.

Methods

Study system and population

The house sparrow (*Passer domesticus*; Fig. 1.1) breeds throughout North America, with the exception of North Central Canada and Greenland (MacGregor-Fors et al., 2019). House sparrows are secondary cavity nesters, they lay a clutch of 3-6 eggs, and they incubate for 11-12 days. House sparrows typically begin incubation after laying the penultimate egg. Both sexes incubate the eggs, and brood and feed the young. Nestlings typically fledge 14 days post-hatching (Anderson, 2006).

For this study, I sampled house sparrows from an established field site in Fargo, North Dakota. These birds breed in nest boxes hung on the sides of buildings owned by the Animal Sciences Department at North Dakota State University. This field site is located less than a mile from the NOAA weather station at Hector International Airport, thus weather and climate data are highly likely to reflect that of the field site macroenvironment.

Nest monitoring, body mass measurements and blood sample collection

Nests were checked daily for laying, and the onset of laying was recorded. Nests were continually monitored until clutch completion, at which point nests were not disturbed after day 2 of incubation to avoid triggering parental abandonment of the nest. On day 10 of incubation, nest monitoring was resumed, and the date that the first nestling hatched was recorded as nest hatch date. Nestlings were individually marked with different colored Sharpie® markers. Around days 2 and 10 post-hatching, body mass measurements were obtained to the nearest tenth using an electronic balance. Some of these birds were also disturbed to be measured around days 6 and 8

for a different study. To measure telomere length, a small blood sample on days 2 and 10 was collected in heparinized capillary tubes via veinpuncture of the brachial vein. Samples were kept on ice in the field for less than 6 hours. Samples were then centrifuged and separated into plasma and red blood cells and were then stored at - 80°C until telomere measurement. Some of these birds also disturbed to give blood samples around day 6 post-hatching for a different study.

As part of a different study (but not in fulfillment of this study's aims), after day 2 post-hatching, some nestlings were placed into treatment groups. In natural variation nests, nestlings were only disturbed to be sampled as previously described. In the stress treatment, half of the nestlings in the nest were removed from the nest for one hour each day and placed in canvas bird bags to simulate parental neglect. The control group consisted of the remaining siblings left in the nest while their siblings were stressed.

Nest microclimate and ambient temperature data

iButton (DS1921G-F5# Thermochron 4k) temperature loggers (n = 60) were placed directly under the nest cup sometime between day 1 and 7 of the onset of incubation (mean $\pm$ s.d. = day 4.7 $\pm$ 1.4 of incubation). iButton temperature loggers remained in the nest to log nest microclimate every three minutes for a total of 3-4 days. If iButtons were removed or kicked out by the parents, the loggers were replaced and it was noted when replacement had occurred. If the nestlings hatched when the iButton was still in the nest, only the data from before hatching was included in the analysis. After completing microclimate data collection, I obtained the raw data using the iButton software and cleaned the data by plotting the values and looking for outliers and removing any datapoints that were logged after parents had removed the iButton. This only occurred in one nest (D21a), and from looking at the plot, I opted to remove all values after the third day, because the thermal profile of the nest appeared abnormal, and this was most likely when

the iButton was removed. I obtained hourly ambient temperatures from the weather station at Hector International Airport from NOAA.

Telomere measurement

I extracted DNA from the stored red blood cell samples using DNA extraction kits and the associated protocol (Macherey-Nagel Nucleospin®). I assessed DNA concentration using a NanoDrop 8000 (Thermo Scientific®). I measured relative telomere length (T/S ratio) using quantitative PCR (Stratagene Mx3000P). For the single copy control gene (standard), I used Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and I ran telomere reactions and GAPDH reactions on different plates, in duplicate. For each qPCR reaction, I used 12.5 µL of SYBR green Master Mix, 0.25 µL of forward and reverse primers, 6 µL of water, and DNA diluted to 3.33 ng/µL.

For GAPDH, the qPCR thermal profile was 10 minutes at 95˚C, then 40 cycles of 30 seconds at 95˚C and 30 seconds at 60˚C. For telomeres, the qPCR thermal profile was 10 minutes at 95˚C, then 27 cycles of 15 seconds at 95˚C, 30 seconds at 58˚C, and 30 seconds at 72˚C. The number of PCR cycles (Ct) required to accumulate enough fluorescent signal in order to cross a specified threshold are Ct values, and Ct values were calculated for each sample. Average Ct values were used to determine the T/S ratio using the the $2^{\Delta\Delta Ct}$ formula where $\Delta\Delta Ct = (C_t^{Telo} - C_t^{GAPDH})sample - (C_t^{Telo} - C_t^{GAPDH})reference$.

A reference sample was serially diluted to produce a standard curve of 40, 20, 10, 5, and 2.5 ng. The standard curve was included on each plate to assess the efficiencies for each plate and ascertain that all the samples were within the bounds of the standard curve. In addition, I included a water sample on each plate to serve as a negative control and one bird's blood sample was used

as a tissue specific reference sample on all plates. The ICC(2,1) of the reactions for house sparrows is 0.88 in this lab, indicating high repeatability across plates.

Statistical analyses

Defining variables of interest

All analyses were conducted in R version 4.0.3 (R Core Team, 2020). I wanted to understand the role of averages, extremes and consistency of thermal micro- (nest temperature) and macroenvironment (ambient temperature) during incubation, as well as date, in shaping growth, telomeres and survival across ontogeny. For growth, I utilized the response variable of incubation period length, which was calculated as the number of days between when the last egg was laid and the first nestling hatched, and it was a proxy for growth rate during incubation. Day 2 body mass was another response variable, and this was defined by how much each nestling weighed two days post-hatching (grams). Because the birds were too difficult to sample right at hatching (as "hatchlings), I used day 2 body mass as a proxy for the resulting accumulation of mass at the end of incubation. Last, the day 10 body mass response variable was how much each nestling weighed ten days post-hatching (grams) in order to determine whether the effects of incubation temperature on growth persist into later development.

For survival, I utilized the response variables of hatching and day 10 survival, which were binary measures of whether or not an individual bird hatched or survived to day 10, respectively. Failure was denoted as a 0, success was denoted as a 1. For telomere length, I examined the response variables of nestling relative telomere length (T/S ratio) at day 2 (as the resulting DNA damage from growth during incubation) and day 10 (to determine whether DNA damage from growth during incubation persisted until later in development).

For the explanatory variables, I considered average, variance, minimum and maximums for both ambient and nest temperatures. For ambient temperature, calculations utilized all NOAA hourly air temperatures from Hector International Airport for the entirety of the incubation period (the time between last laid egg and first hatched chick), and for nest temperature, calculations utilized all values logged by the iButton for the duration of iButton placement. Average was simply the mean of all values obtained from NOAA (for average ambient temperature) and the iButtons (for average nest temperature). For variance, I utilized standard deviation around the mean squared. Minimum was the single lowest value of all values logged by NOAA (minimum ambient temperature) and the iButtons (minimum nest temperature). Maximum was the single highest value of all values logged by NOAA (maximum ambient temperature) and the iButtons (maximum nest temperature). I also wanted to consider date effects as a proxy for seasonal changes in resource availability, and I used day 1 of incubation in Julian day format. Table A.1. summarizes each explanatory and response variable of interest.

Model building

When I conducted Pearson's Product-Moment Correlations between my variables of interest (Table A.2), my averages and extremes were all highly correlated with each other, and they were also highly correlated with date (Table A.2; Fig. 1.2-1.4), indicating that these variables could not be included as separate fixed effects within the same models without resulting variance inflation. For my consistency measures, variance in ambient temperature exhibited a slight negative correlation with date (Table A.2; Fig. 1.2b), but this correlation was not sufficiently high to justify including it in a separate model. Variance in ambient temperature and variance in nest temperature were not correlated (Table A.2), and date was not correlated with variance in nest

temperature either (Table A.2), which suggested that all of these variables could be included in the same model.

In light of these findings, I opted to conduct a principal components analysis (PCA; R 4.0.3 package: factoextra) for a single seasonal thermal profile variable, comprised of average ambient temperature, average nest temperature, minimum ambient temperature, minimum nest temperature, maximum ambient temperature and maximum nest temperature, as well as date, in order to capture the effects of temperature fluctuations in macro- and microenvironment across the season. The PCA returned seven principal components (PCs; Table A.3). PC1 explained 74.9% of the variation, with similar loadings for each trait (Table A.3). A plot of trait contributions towards PC1 and PC2 (Fig. A.1) is included for reference. However, I chose to include only the predicted values of PC1 in my analyses.

Before day 2 post-hatching, all birds were considered to be part of the natural variation group, and treatment was not included as an effect in any of the statistical models. However, because some birds were placed into treatment groups as part of a different study, I needed to account for possible effects of the stress and control treatments on the response variables of interest. After day 2 post-hatching, treatment effects were included in the statistical models if found significant. Day 10 telomere length did not vary with treatment and neither did day 10 survival, but there was a significant effect for day 10 mass (Table A.2).

In addition to the effects of temperature seasonal thermal profile (PC1), temperature consistency (variance in ambient temperature and variance in nest temperature) and treatment (for day 10 body mass), I also included the explanatory variables of clutch size for early developmental outcomes (incubation period length, hatching survival, day 2 body mass and day 2 telomere length), brood size for late developmental outcomes (day 10 body mass, day 10 telomere length

and day 10 survival) and assay (for day 2 and day 10 telomere lengths), since these variables could also potentially explain variation in the response variables of interest.

Conducting the analysis

For the linear mixed effect models (response variables: day 2 body mass, day 2 telomere length, day 10 body mass and day 10 telomere length; R 4.0.3 package: nlme and MASS) and binomial generalized linear mixed effect models (response variables: hatching survival and day 10 survival; R 4.0.3 packages: lme4, lmerTest and glmm), all these variables were fit as fixed effects, and I also included nest as a random effect, as multiple individuals from the same nest were included in the analysis. I conducted a linear model for incubation period length as there was only one measure per nest. Table A.4 summarizes the complete list of models utilized. I calculated model R^2 for the linear mixed effect and binomial generalized linear mixed effect models using the rsq package in R 4.0.3 and used the adjusted R^2 in the summary output for the linear models.

Figure 1.1. Photograph of adult male (left) and a fledgling (right) house sparrow (Jones & Hendry, n.d.).

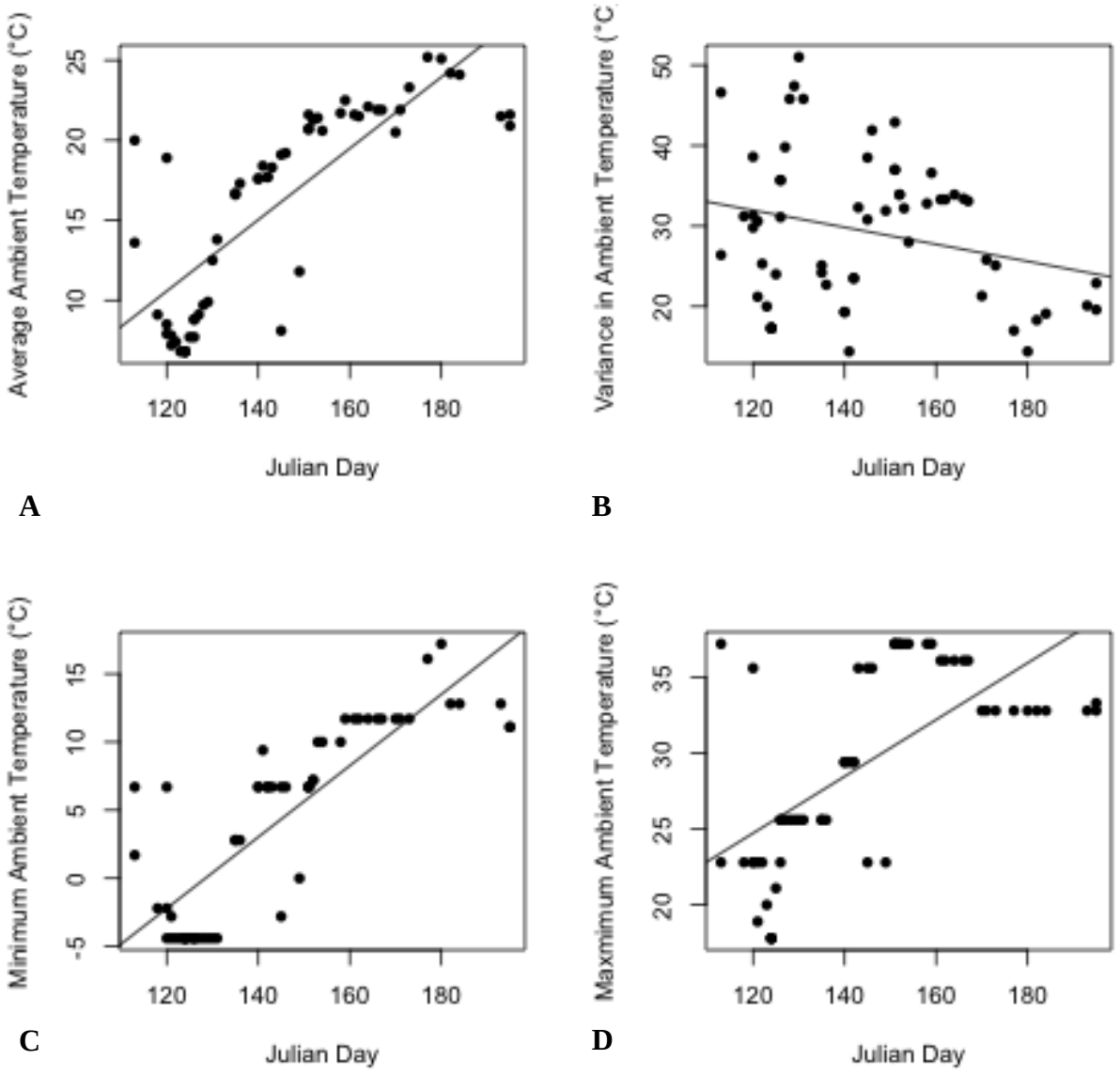

Figure 1.2. a) Average ambient temperature plotted against Julian day (n = 60), b) Variance in ambient temperature plotted against Julian day (n = 60), c) Minimum ambient temperature plotted against Julian day (n = 60), d) Maximum ambient temperature plotted against Julian day (n = 60).

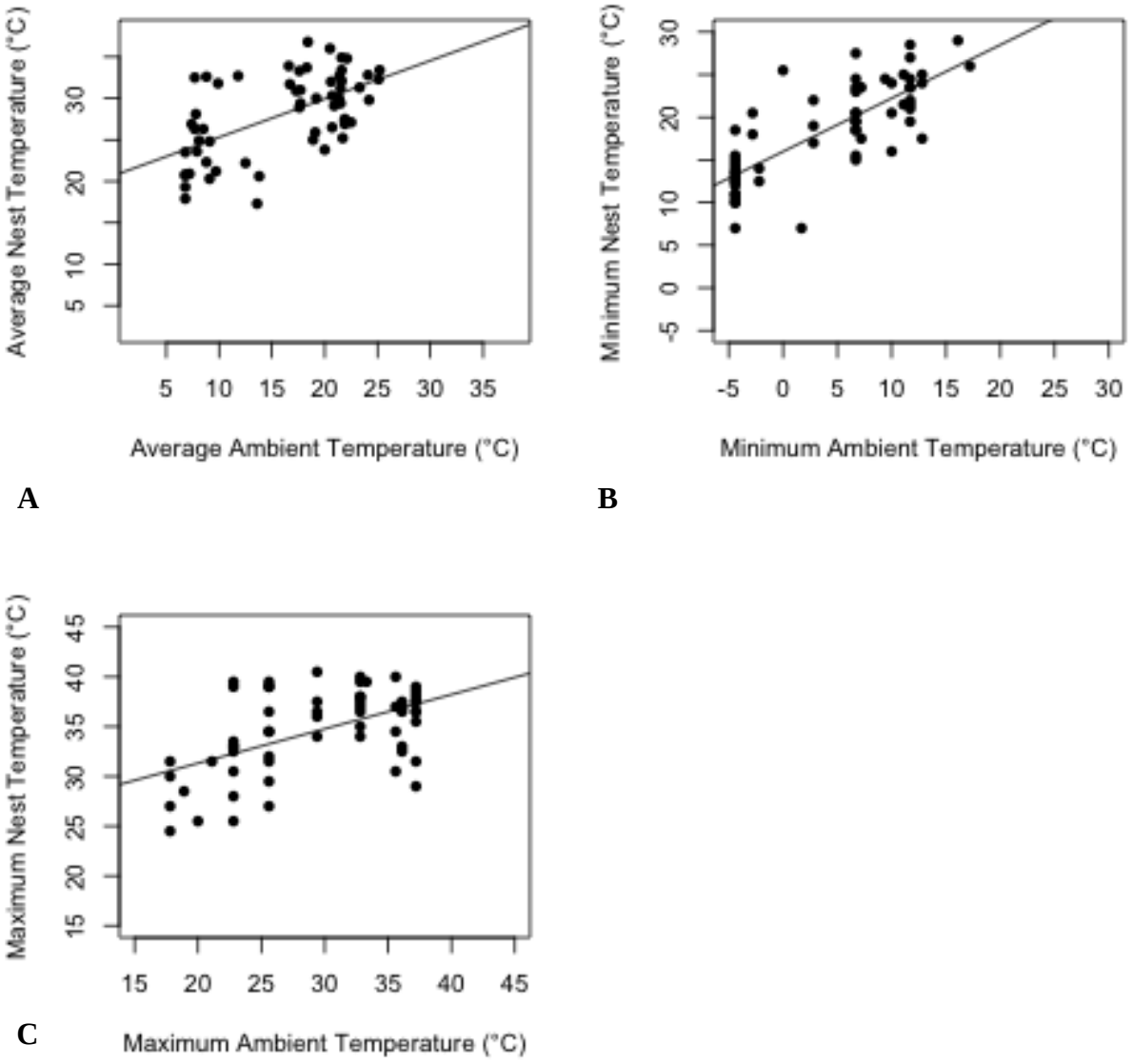

Figure 1.3. a) Average nest temperature plotted against average ambient temperature (n = 60), b) minimum nest temperature plotted against minimum ambient temperature (n = 60), c) maximum nest temperature plotted against maximum ambient temperature (n = 60).

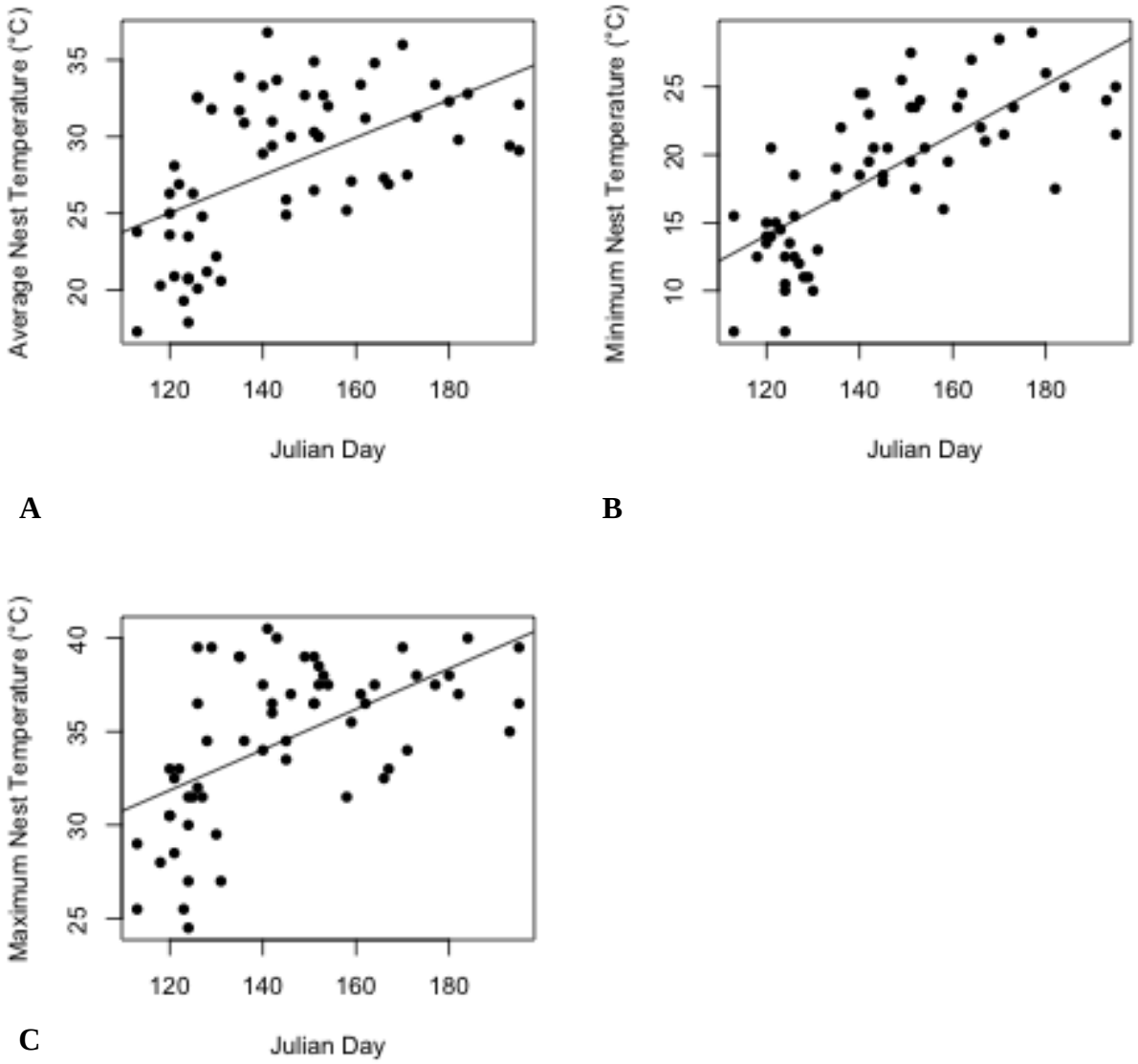

Figure 1.4. a) Average nest temperature plotted against Julian day (n = 60), b) Minimum nest temperature plotted against Julian day (n = 60), c) Maximum nest temperature plotted against Julian day (n = 60).

Results

Effects of incubation temperature, seasonal thermal profile and consistency on incubation period length and body mass at days 2 and 10

Incubation period length increased with the predicted values of PC1 (Estimate $\pm$ SE = 0.21 $\pm$ 0.067, t = 3.22, df = 55, p = 0.0021; Fig. 1.5a). Due to the negative trait loadings of PC1, this indicates that incubation period length decreased with date and the temperature metrics of interest.

Incubation period length increased with variance in ambient temperature (Estimate ± SE = 0.039 ± 0.014, t = 2.62, df = 55, p = 0.011; Fig. 1.5b) and variance in nest temperature (Estimate ± SE = 0.057 ± 0.028, t = 2.00, p = 0.049; Fig. 1.5c), but did not vary with clutch size (Table 1.1).

PC1 negatively predicted body mass for day 2 (Value ± SE = - 0.25 ± 0.090, t = - 2.87, df = 51, p = 0.0059; Fig. 1.6), and day 10 (Value + SE = - 0.76 + 0.34, t = - 2.21, df = 38, p = 0.033; Fig. 1.7), and due to the negative trait loadings of PC1, this indicates that day 2 and 10 body mass increase with date and the temperature metrics of interest. Day 2 and day 10 body mass did not vary with any other explanatory variables of interest (Table 1.1)

Effects of seasonal thermal profile, thermal consistency mass on telomere length at day 2 and 10

Day 2 telomere length did not vary with any explanatory variables of interest, and with the exception of assay, neither did day 10 telomere length (Table 1.1). Incubation period length and day 2 telomere length exhibited a weak positive correlation (r = 0.14, t = 2.08, df = 204, p = 0.038), There was no correlation between day 2 mass and telomere length (r = 0.035, t = 0.050, df = 204, p = 0.61), or between day 10 mass and telomere length (r = 0.027, t = 0.31, df = 36, p = 0.75).

Effects of incubation temperature, seasonal thermal profile and consistency on hatching and day 10 survival

Hatching survival decreased with variance in nest temperature (Estimate ± SE = - 0.077 ± 0.032, z = - 2.39, df.resid = 291, p = 0.016; Fig. 1.8), but did not vary with other independent variables included in the model (Table 1.1). No explanatory variables of interest predicted day 10 survival (Table 1.1).

Table 1.1. Results of the statistical analysis.

Response Variable	Effects	Estimate	SE	t value	DF	p value
Incubation	(Intercept)	9.67	1.043	9.27	55	< 0.0001 *
period length	Pc1	0.21	0.067	3.22	55	**0.0021 ***
	Variance in ambient temperature	0.039	0.014	2.62	55	**0.011 ***
Adj. R^2 = 0.35	Variance in nest temperature	0.057	0.028	2.00	55	**0.049 ***
	Clutch size	-0.079	0.16	-0.48	55	0.62
Response Variable	Effects	Value	SE	t value	DF	p value
Day 2 body	(Intercept)	4.36	1.37	3.17	159	0.0018 *
mass	Pc1	-0.25	0.090	-2.87	51	**0.0059 ***
	Variance in ambient temperature	0.024	0.019	1.22	51	0.22
Model R^2 = 0.42	Variance in nest temperature	0.074	0.043	1.71	51	0.092
	Clutch size	-0.060	0.21	-0.28	51	0.77
Day 10 body	(Intercept)	23.53	4.12	5.69	114	< 0.0001 *
mass	Pc1	-0.76	0.34	-2.21	38	**0.033 ***
	Variance in ambient temperature	0.019	0.076	0.24	38	0.80
Model R^2 = 0.74	Variance in nest temperature	0.00452	0.16	0.027	38	0.97
	Treatment: natural variation	-2.42	1.46	-1.65	38	0.10
	Treatment: stress	-0.66	0.53	-1.23	114	0.21
	Brood size	-0.33	0.64	-0.52	38	0.60
Day 2 telomere	(Intercept)	0.38	0.47	0.80	129	0.41
length	Pc1	0.040	0.028	1.43	51	0.15
	Variance in ambient temperature	0.0080	0.0058	1.36	51	0.17
Model R^2 = 0.62	Variance in nest temperature	-0.010	0.011	-0.87	51	0.38
	Clutch size	0.074	0.054	1.38	51	0.17
	Assay 2	-0.28	0.32	-0.86	129	0.38
	Assay 3	-0.11	0.37	-0.30	129	0.76
	Assay 4	0.36	0.30	1.17	129	0.24
	Assay 5	0.076	0.32	0.23	129	0.81
	Assay 6	-0.16	0.31	-0.54	129	0.58
	Assay 7	0.063	0.30	0.20	129	0.83
	Assay 8	0.31	0.31	0.99	129	0.32
	Assay 9	0.20	0.32	0.64	129	0.51
	Assay 10	0.020	0.31	0.065	129	0.94
	Assay 11	0.54	0.31	1.71	129	0.088
	Assay 12	-0.025	0.30	-0.085	129	0.93
	Assay 13	0.087	0.32	0.26	129	0.78
	Assay 14	0.24	0.34	0.69	129	0.48
	Assay 15	-0.21	0.27	-0.77	129	0.43
	Assay 16	-0.0011	0.27	-0.0043	129	0.99
	Assay 17	-0.13	0.27	-0.47	129	0.63

Table 1.1. Results of the statistical analysis (continued).

Response Variable	Effects	Value	SE	t value	DF	p value
	Assay 18	0.012	0.26	0.044	129	0.96
	Assay 19	0.21	0.27	0.78	129	0.43
	Assay 20	0.21	0.28	0.73	129	0.46
	Assay 21	0.18	0.28	0.63	129	0.52
	Assay 22	-0.023	0.28	-0.083	129	0.93
Day 10 telomere length Model R^2 = 0.60	(Intercept)	0.57	0.36	1.55	80	0.12
	Pc1	-0.022	0.025	-0.87	33	0.39
	Variance in ambient temperature	0.0028	0.0057	0.49	33	0.62
	Variance in nest temperature	0.011	0.011	1.00	33	0.32
	Brood size	0.0080	0.047	0.16	33	0.86
	Assay 2	-0.27	0.24	-1.15	80	0.25
	Assay 3	-0.0056	0.19	-0.028	80	0.97
	Assay 4	0.93	0.23	3.92	80	**0.00020 ***
	Assay 5	0.16	0.24	0.67	80	0.50
	Assay 6	0.24	0.23	1.06	80	0.29
	Assay 7	0.25	0.23	1.06	80	0.28
	Assay 8	0.36	0.24	1.48	80	0.14
	Assay 9	-0.054	0.25	-0.21	80	0.83
	Assay 10	0.076	0.23	0.32	80	0.74
	Assay 11	0.76	0.26	2.90	80	**0.0048 ***
	Assay 12	0.063	0.22	0.27	80	0.78
	Assay 13	0.14	0.24	0.57	80	0.56
	Assay 14	0.13	0.30	0.43	80	0.66
	Assay 15	0.12	0.22	0.57	80	0.56
	Assay 16	0.55	0.22	2.43	80	**0.017 ***
	Assay 17	0.30	0.21	1.39	80	0.166
	Assay 18	0.44	0.22	1.99	80	**0.049 ***
	Assay 19	0.41	0.22	1.81	80	0.073
	Assay 21	0.60	0.32	1.86	80	0.066
	Assay 22	0.39	0.22	1.72	80	0.089
Response	Effects	Estimate	SE	z value	DF.r^+	p value
Hatching survival Model R^2 = 0.10	(Intercept)	0.92	1.24	0.73	291	0.45
	Pc1	0.10	0.079	1.30	291	0.19
	Variance in ambient temperature	-0.0050	0.017	-0.29	291	0.77
	Variance in nest temperature	-0.077	0.032	-2.39	291	**0.016 ***
	Clutch size	0.22	0.19	1.18	291	0.23
Day 10 survival	(Intercept)	-0.041	2.38	-0.01	209	0.98
	Pc1	-0.13	0.18	-0.72	209	0.46
	Variance in ambient temperature	-0.053	0.044	-1.20	209	0.22

Table 1.1. Results of the statistical analysis (continued).

Response	Effects	Estimate	SE	z value	DF.r[+]	p value
Model $R^2 = 0.54$	Variance in nest temperature	0.041	0.094	0.44	209	0.65
	Brood size	0.68365	0.36	1.86	209	0.061

[+] DF.r = residual degrees of freedom

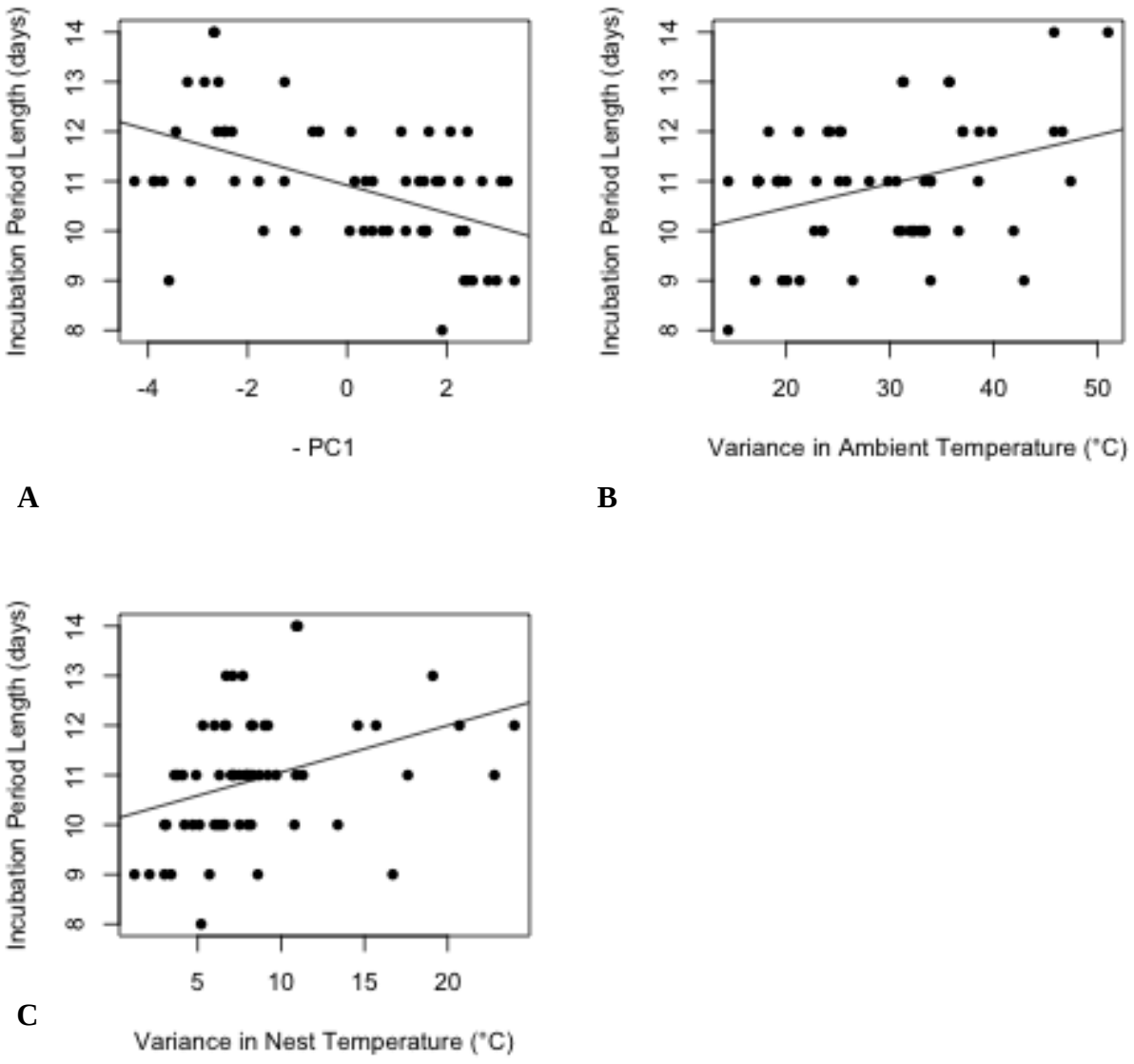

Figure 1.5. a) Incubation period length plotted against predicted values of -PC1 (n = 60), b) Incubation period length plotted against variance in ambient temperature (n = 60), c) Incubation period length plotted against variance in nest temperature (n = 60).

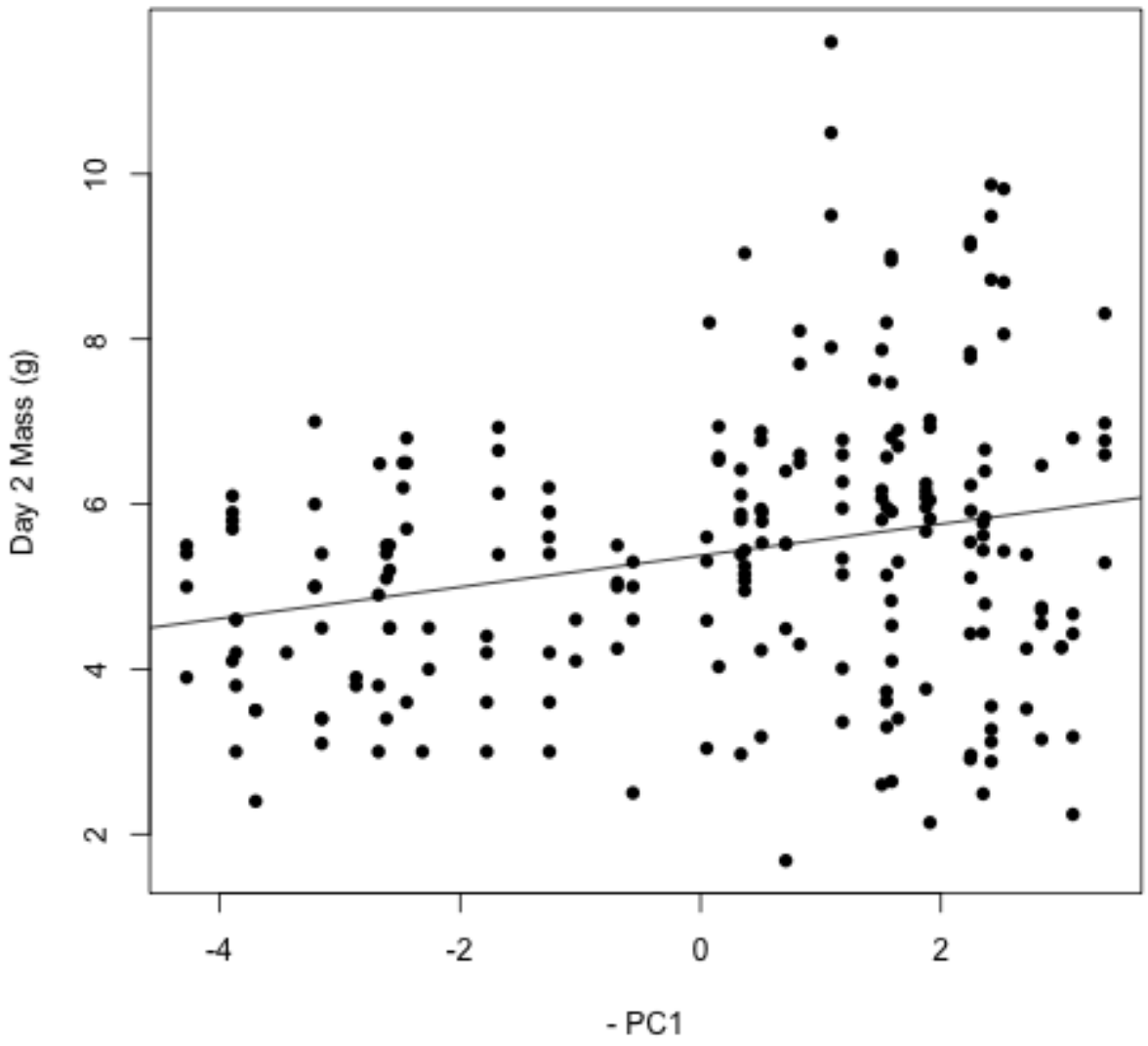

Figure 1.6. Day 2 mass plotted against -PC1 (n = 215 birds from n = 56 nests).

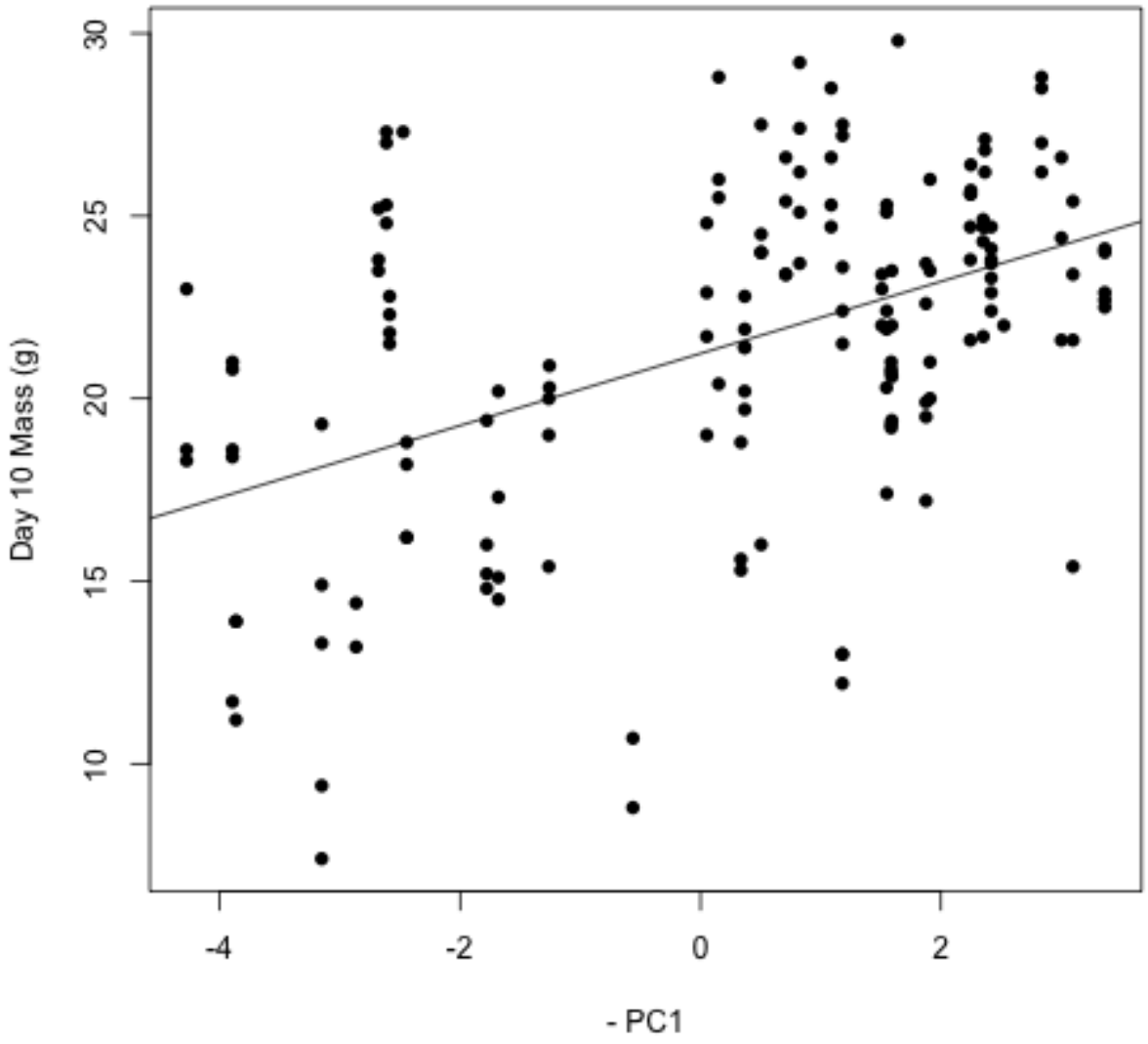

Figure 1.7. Day 10 mass plotted against -PC1 (n = 159 birds from n = 44 nests).

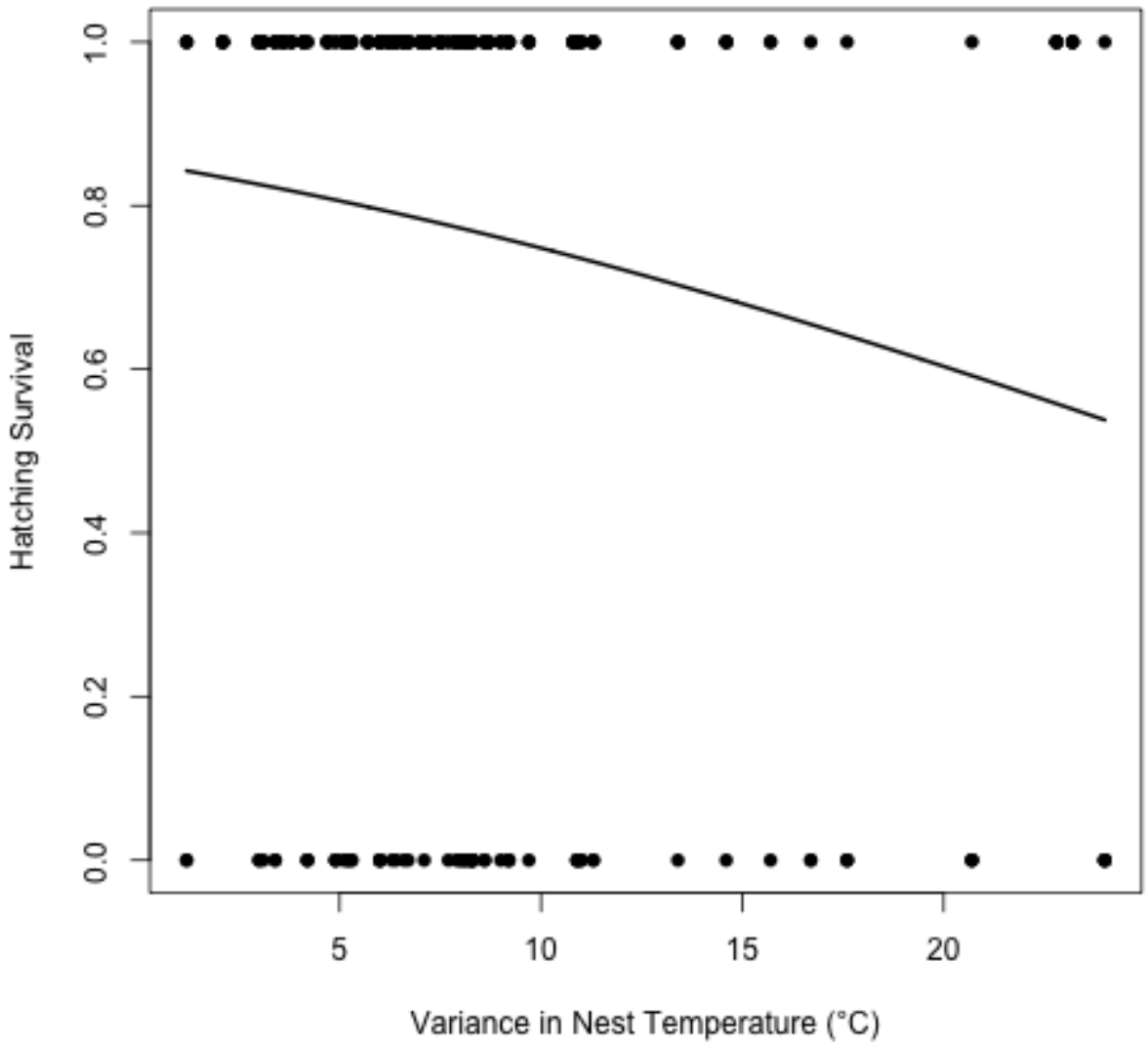

Figure 1.8. Hatching survival plotted against variance in nest temperature (n = 297 birds from n = 60 nests).

Discussion

This study sought to shed light on how variance, averages and extremes in nest and ambient temperature, as well as date can influence in developmental outcomes in the house sparrow by logging naturally varying developmental conditions and pairing those conditions with developmental rate and body mass, telomere length and survival across ontogeny. The results of this study indicate that seasonal thermal profile and consistency of thermal environment can have growth and survival implications for developing house sparrows, but these effects are largely

context-dependent, as mass decreased with seasonal thermal profile (encompassing averages and extremes of nest and ambient temperature as well as date), while hatching survival decreased with variance in nest temperature, and incubation period length varied with all metrics. Field integrative biologists should consider moving away from "the golden mean" (Williams, 2008) and focusing more on measures of variability in order to characterize the implications for developmental outcomes linked to fitness.

Temperature seasonal thermal profile and consistency predict incubation period length, but only seasonal thermal profile in temperature influences body mass at day 2 and 10

Higher temperatures increased developmental rate during incubation of house sparrows, consistent with the findings in the literature for both altricial (Ospina et al., 2018) and precocial (Nord & Nilsson, 2021; Vedder et al., 2018) birds. Generally, warmer temperatures increase developmental rate because biochemical reactions promoting growth occur at a faster rate (Vedder et al., 2018), and low incubation temperatures increase energetic demand for developing embryos, thereby increasing incubation period length (Rubin et al., 2021; Nord & Nilsson, 2021). Birds incubated at colder conditions tend to have less residual yolk at hatching in comparison to birds incubated at warmer conditions, indicative of a need to stretch a finite amount of resources across a longer period of time (Olson et al., 2006; zebra finch; Wada et al., 2015).

Higher temperatures led to an increase in body mass at day 2. This is consistent with the findings in the literature, as cold-incubated birds were smaller at hatching (Nord & Nilsson, 2021; Vedder et al., 2018) and heated chicks were heavier at hatching (Carvalho et al., 2020). With slower embryonic growth, birds experiencing colder conditions likely exhibited a decreased the rate of cell divisions (Vedder et al., 2018), so birds in warmer conditions likely exhibited more rapid cell divisions, allowing them to accumulate greater mass in a shorter period of time. The

seasonal thermal profile effect for body mass at day 10 is more difficult to interpret. Body size effects of incubation temperature can persist until late into postnatal development (the period after hatching and before the bird leaves the nest to fledge; Ospina et al., 2018). However, as this is a natural variation study, it is unclear whether this effect is a result of incubation or postnatal conditions. Ambient conditions, microclimate, parental attentiveness during both incubation and postnatal growth, and nestling provisioning by parents during postnatal growth could all potentially influence day 10 body mass. Future studies should seek to clarify the relative contributions of thermal environment and nestling feeding in shaping late postnatal phenotypes.

Variance in both ambient and nest temperature positively predicted incubation period length, indicating that greater inconsistency slows embryonic growth rates. The literature also supports these findings, as increased variability can lead to a lower average temperature than a more constant warm temperature, so the results of inconsistent conditions are typically similar to the results of constant low temperature conditions (Stier et al., 2020; Rubin et al., 2021). Short exposure to these cooler temperatures may have been sufficient to developmentally program these birds to increase energy usage in preparation for thermoregulation in an unstable environment (Nord & Nilsson, 2021).

Body mass at day 2 did not vary with variance in ambient or nest temperature, which is consistent with findings in the literature, in which periodic cooling of eggs did not influence hatchling mass outcomes (Rubin et al., 2021). This may be because there are limits to how small a bird can be at hatching in order to successfully pip and hatch from the egg (Rubin et al., 2021), and this may be especially true for an altricial species. If highly inconsistent developmental conditions did influence hatchling/early postnatal mass (as in Stier et al. 2020), perhaps those birds failed to survive to hatching.

Microclimate temperature consistency predicts hatching survival, but day 10 survival does not vary with seasonal thermal profile or consistency

Hatching survival did not vary with seasonal thermal profile. In the literature, incubation temperatures averaging 38.4°C can reduce hatching survival in zebra finches (Wada et al., 2015). House sparrows are artificially incubated between average temperatures of 37.2°C and 37.8°C (Cooper et al., 2011; Wetherbee & Wetherbee, 1961), and the field nest temperature averages in this study rarely rose above 35°C (although maximum nest temperatures did on occasion). It is possible that the field conditions did not reach levels severe enough and for sufficient duration to attenuate hatching survival.

Variance in nest temperature negatively predicted hatching survival. This indicates that birds developing under highly inconsistent nest conditions are less likely to survive to hatching. These results are slightly puzzling considering that hatching survival was not impacted by thermal variability in other studies (Stier et al., 2020; Rubin et al., 2021). However, in both of these studies, thermal variability was induced by decreasing temperature for thirty minute intervals a few times a day. In Rubin et al. 2021, the variance in incubation temperature in the periodically cooled group was 3.02°C. In the field in this study, variance in nest temperatures across incubation reached 20°C in some nests. While it is possible that parents in the field may have counteracted some of these effects via incubation behavior, the magnitude of this variance may explain why hatching survival effects are observed in this study but not in others.

Nest microclimate is impacted by both ambient temperature and parental incubation behavior, which may explain why variance in nest and ambient temperature are not correlated, and why only variance in nest temperature predicts hatching survival. Parents play a role in creating a consistent microenvironment by increasing their attentiveness and preventing egg cooling (Clauser

& McRae, 2017; Conway & Martin, 2000). Parental behavior may underly variation in nest temperature, as more attentive parents can in theory more tightly regulate nest conditions. It is also possible that parents with higher attentiveness had higher hatching success. Parents with high attentiveness should create a more consistent developmental environment (Clauser & McRae, 2017), and prevent egg cooling (Conway & Martin, 2000) and exposure to detrimental conditions. Future studies examining parental incubation behavior will be necessary to test this hypothesis.

Day 10 survival did not vary with any ambient or nest temperature measures. In both zebra finches (Wada et al., 2015) and American robins (Ospina et al., 2018), exposure to low incubation temperatures decreased post-hatch survival. However, in this study, there was little variation in day 10 survival (as most birds that hatched also survived to day 10), so it is possible that any temperature effects could not be detected.

Telomere length at days 2 and 10 do not vary with consistency or seasonal thermal profile in temperature

Day 2 and day 10 telomere lengths did not vary with any ambient and nest temperature measure. This finding is puzzling in light of the positive associations between temperature seasonal thermal profile and day 2 and 10 body mass. In theory, telomeres shorten with rapid cell divisions, so I expected that telomeres would be shorter in the presence of faster growth rates. I did find a weak positive correlation between incubation period length and day 2 telomere length, but not between body mass and telomere length for days 2 and 10. Regarding growth rates associated with high temperatures and highly variable conditions, patterns of shorter telomeres in the presence of high temperatures and thermal variability are reflected in the literature (Stier et al., 2020; Vedder et al., 2018).

However, it is worth noting that most studies have examined telomeres in precocial and semi-precocial birds, but house sparrows are altricial. Generally, telomerase activity declines at the end of the embryonic period (Stier et al., 2020); however, this has not been established in altricial bird species such as house sparrows. Altricial birds have an incredibly high postnatal growth rate, meaning that without the expression of telomerase or some other mechanism (such as ALT; Stier et al., 2020) in place to elongate telomeres, telomere length would likely decrease rapidly during development. It is possible that telomerase and/or components of a certain telomere elongation pathway are expressed throughout the postnatal period in house sparrows, explaining why shorter telomeres were not observed even in the presence of rapid cell division.

Implications and future directions

The results of this study indicate that the effects of seasonal thermal profile and consistency on developmental outcomes are context dependent. However, the sources of microclimate seasonal thermal profile and consistency are not entirely clear. Future studies should seek to parse out the relative contributions of postnatal conditions and incubation conditions in shaping later phenotypic consequences, as well as the relative contributions of food availability and temperature.

CHAPTER 3. PARENTAL BEHAVIOR AND AMBIENT TEMPERATURE ACROSS ONTOGENY SHAPE NESTLING TRAITS IN A CONTEXT-DEPENDENT MANNER IN THE HOUSE SPARROW (*PASSER DOMESTICUS*)

Introduction

Understanding the longitudinal fitness effects of early developmental plasticity has become increasingly important in the midst of rapid environmental changes associated with climate change. Altricial birds transition from ectothermy (gaining the majority of their body heat from the environment) to endothermy (gaining the majority of their body heat from metabolic processes; McCue, 2004; Tzschentke & Rumpf, 2011) approximately five days post-hatching (Andrew et al., 2017). This indicates that during incubation (embryonic development in the egg) and early postnatal development (after hatching and until leaving the nest to fledge), these birds are vulnerable to environmental temperatures.

Artificial incubation studies reveal the myriad of developmental consequences incubation temperature can have for birds during both incubation and postnatal growth. Exposure to low incubation temperatures can slow developmental rate and decrease body size (Nord & Nilsson, 2021; Japanese quail), and high temperature postnatal growth conditions can promote decreased body mass in comparison to birds reared at lower temperatures (Andrew et al., 2017; zebra finch). Last, periodic egg cooling can lead to slow post-hatch growth as a result of those inconsistent conditions (Rubin et al., 2021; zebra finch). Exposure to very high temperatures can attenuate hatching survival (Wada et al., 2015; zebra finch; Carvalho et al., 2020; Stier et al., 2020; Japanese quail), and both high and fluctuating incubation temperature can decrease telomere length (Stier et al., 2020).

Telomeres are protective complexes located at the end of chromosomes, and they shorten with number of cell divisions (Vedder et al., 2018; Stier et al., 2020), in part because of the "end-replication problem" (Stier et al., 2020), in which DNA polymerase cannot fully replicate the linear ends of DNA (Levy et al., 1992) during DNA replication. Increased oxidative stress as a result of reactive-oxygen species (ROS) produced during cellular respiration (Yubero-Serrano et al., 2014) can also attenuate telomeres (Stier et al., 2020). There are mechanisms in place to restore and elongate telomeres (such as the expression of telomerase and the Alternative Lengthening of Telomeres pathway), but activity most often decreases in adult somatic tissues (Stier et al., 2020).

Averages and consistency of ambient temperature can be especially important in shaping early developmental outcomes such as telomeres, directly and indirectly. Nest microclimate determines the thermal profile birds experience during development, and it is dictated by a combination of ambient temperature conditions, solar radiation, gas composition, humidity and solar radiation (Heenan, 2013). How nests are constructed and how well insulated they are can be especially important in shaping reproductive performance in cavity nesting birds (Akresh et al. 2017), and a well-chosen sheltered nest site can help counteract convective heat loss in cavity nesting birds (Heenan, 2013). Averages in nest temperature are positively correlated with ambient temperature in North Dakota house sparrows (Dennis, 2022 [unpublished]). Parental incubation behavior plays an important role in maintaining consistent nest thermal conditions by warming the eggs (Clauser & McRae, 2017). During early postnatal development, parents brood their ectothermic chicks to prevent them from cooling (Oswald et al., 2021), and this decreases as nestlings begin transitioning to endothermy (Yoon et al., 2016). Parents tightly regulate developmental temperature during incubation and early postnatal growth via behavior, but parental off-bouts expose their developing young to nest microclimate conditions.

More indirectly, ambient temperature can influence parental incubation and brooding behavior. Birds such as king rails stand over their eggs to shade them in the presence of high temperatures, and some ground nesters hover over their eggs (Clauser & McRae, 2017). In wood ducks with experimentally reduced down nest microclimates, females shortened morning recesses and increased incubation constancy to mitigate nest cooling (McClintock et al., 2014). As temperatures warm, yellow warbler females spend less time on the nest (Rohwer & Purcell, 2019), and orange-crowed warbler parents in colder climates often take shorter off-bouts to avoid egg cooling (Conway & Martin, 2000). In Cape rockjumpers, parents spend less time brooding in the presence of high maximum temperatures (Oswald et al., 2021). However, parental care is costly to parents in general (Yoon et al., 2016). For instance, uniparental incubators such as female eiders may lose up to 23-46% of their body mass as a result, and incubating birds can have a 20-50% higher metabolic rate than non-incubating birds (DuRant et al., 2013). Incubation in the presence of extreme temperatures can be especially costly for parents (Vleck, 1981). Further, by engaging in any form of parental behavior, parents lose out on time to forage for themselves or take part in other forms of self-maintenance (Álvarez & Barba, 2014). And as global temperatures rise, parents may have to choose between the care of their offspring and their own self-maintenance as well as their own future reproductive potential (DuRant et al., 2019).

Postnatal ambient temperature can also indirectly influence developmental outcomes via parental feeding behavior and resource availability. In species such as Cape rockjumpers, parents decrease feeding trips with increased ambient temperature (Oswald et al., 2021). At the late nestling stages, birds have begun the transition to endothermy, so while they are less dependent on parental brooding, they require energy (and therefore, more food) to thermoregulate in the presence of cold temperatures. Parents may attempt to offset the effects of cold temperatures by increasing

provisioning, but they are constrained by prey availability, and prey abundance can also decrease in the presence of low temperatures when the nestlings need it most (Yoon et al., 2016). For instance, aerial insectivores such as tree swallows rely upon actively flying insects to feed their young, and abiotic factors such as temperature influence insect flight patterns. Therefore, exposure to sudden cold snaps can lower prey availability, leading to decreased parental provisioning, and as a result attenuated nestling survival (Shipley et al., 2020). Low abundance of insects is associated with high rates of house sparrow nestling starvation and low fledgling body mass, decreasing the likelihood of recruitment as a breeding adult (Peach et al., 2015).

While previous work has examined the role of temperature in shaping developmental outcomes across incubation and postnatal growth, as well as the role of temperature in shaping parental behavior, few studies have considered the relative contributions of ambient temperature and parental behavior across developmental stages in shaping growth trajectories and fitness consequences. The aim of this study was to determine which factors (ambient temperature and/or parental behavior during incubation and postnatal growth) drive developmental outcomes to the greatest extent in early- and late-stage nestlings, in an altricial species that engages in biparental care, the house sparrow (*Passer domesticus*).

I hypothesized that parents modify behavior in response to ambient temperature in order to modulate developmental environment and maximize fitness outcomes, and parents who successfully maintain tight regulation of the developmental environment through increased attentiveness have the best nestling outcomes. Because female house sparrows have warmer abdomens than males do (Bartlett et al., 2005), I predicted that female behavior influences developmental outcomes more than male behavior does. I also predicted that birds developing under cold conditions would have lower hatching and day 10 survival, later hatching, smaller body

size and longer telomeres. However, parents with high incubation and postnatal attentiveness and feeding rates would produce nestlings with generally better developmental outcomes, although high feeding rates could decrease offspring telomere length.

Methods

Study system and population

The house sparrow (*Passer domesticus*; Fig. 2.1) is a secondary cavity nester (Anderson, 2006) found throughout the contiguous United States (MacGregor-Fors et al. 2019). House sparrows are biparental incubators and brooders who generally lay 3-6 eggs. Hatching generally occurs after 11-12 days and nestlings typically fledge at about 14 days post-hatching (Anderson, 2006). This study utilized house sparrows that breed in nest boxes at an established field site in Fargo, North Dakota. The field site consists of nest boxes hung on sides of North Dakota State University Animal Sciences Department buildings, and the site is located less than one mile away from the NOAA weather station at Hector International Airport, from where I obtained hourly ambient temperatures for this study.

Nest filming during incubation and postnatal growth

During the summer of 2020, video cameras were set up in the field facing the nest boxes starting between the hours of 6:00am to 7:30am. For each nest, one video was filmed during incubation, and one video was filmed during postnatal growth. Incubation videos were filmed around day 6 of incubation and postnatal videos about 6 days after the first nestling in the nest hatched to control for developmental stage of the nestlings. Cameras were placed at least 0.5 meters from the nest (Schaefer, 2004), and the entirety of the nest box entryway was visible in each video frame, and the videos ran for 4 hours and 10 minutes. Sometimes, the camera was knocked over by the wind, rain prevented visibility, or the camera ran out of battery or memory

before completing filming. In these cases, I truncated the videos and only included the usable footage in the analysis. The nest, date of filming and time the video started were all noted by the person recording.

Nest monitoring, body mass measurements and blood sample collection

Nests were monitored daily for the onset of laying and clutch completion. To avoid triggering parental abandonment of focal nests, nests were not disturbed after day 2 of incubation. Nest monitoring resumed on day 10 of incubation until the first bird hatched, as hatching typically occurs after 11-12 days (Anderson 2006). Hatchlings were marked with different colored Sharpie® markers in order to distinguish between them. After day 2 post-hatching, some nestlings were placed into treatment groups as part of a different study (but not in fulfillment of this study's aims). In natural variation nests, nestlings were only disturbed to be sampled. For most birds, body mass was measured to the nearest tenth using an electronic balance and blood samples were collected in heparinized capillary tubes by puncturing the bird's brachial vein around days 2 and 10 post-hatching. Blood samples then remained on ice until they were centrifuged and separated into plasma and red blood cells, which were stored at - 80°C until telomere measurement. Some birds were also disturbed to be bled around day 6 and also measured around days 2, 4, 6 and 8. In the stress treatment, half of the nestlings were removed from the nest for one hour each day and kept in canvas bird bags to simulate parental neglect. The control group consisted of the siblings remaining in the nest while their siblings were stressed.

Watching the videos

I used the program BORIS (Friard & Gamba, 2016) to watch the incubation and nestling rearing videos. For the ethogram (Table A.5), I noted when the male and female entered and exited the nest, how long they stayed fully in the box (remaining), and the amount of time the bird spent

in the entryway (departing). For incubation, I assumed that time spent remaining was incubating (see Kopisch et al., 2005), but I did not count departing behavior when calculating incubation attentiveness. It is worth noting that including departing in the calculation did not change the results. For the postnatal period, I counted each time the parent entered the box as a provisioning trip (postnatal visit), and extended time spent remaining was assumed to be brooding (postnatal attentiveness).

Telomere measurement

I extracted DNA for telomere measurement from the stored red blood cell samples using DNA extraction kits and the protocol written by the manufacturer (Macherey-Nagel Nucleospin®). I assessed DNA concentration using a NanoDrop 8000 (Thermo Scientific®) and then measured relative telomere length (T/S ratio) using quantitative PCR (Stratagene Mx3000P). I used Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the single copy control gene (standard), and telomere and GAPDH reactions were always run in duplicate on different plates. Each of these qPCR reactions consisted of 12.5 µL of SYBR green Master Mix, 6 µL of water, 0.25 µL of forward and reverse primers and DNA extracted from the blood sample diluted to 3.33 ng/µL.

I serially diluted a reference sample to create a standard curve of 40, 20, 10, 5, and 2.5 ng. This standard curve was included on each plate to ensure that all of the samples were within the bounds of the standard curve and determine the efficiencies for each plate. In addition, on all plates, I included one bird's blood sample was used as a tissue specific reference sample and a water sample on each plate as a negative control.

For telomeres, the qPCR thermal profile used was 10 minutes at 95˚C, 27 cycles of 15 seconds at 95˚C, 30 seconds at 58˚C, and 30 seconds at 72˚C. For GAPDH, it was 10 minutes at

95˚C, 40 cycles of 30 seconds at 95˚C and 30 seconds at 60˚C. Ct values, the number of PCR cycles (Ct) required to accumulate sufficient fluorescent signal to cross a determined threshold, were calculated for each sample, and average Ct values were used to determine the T/S ratio. This calculation used the $2^{\Delta\Delta Ct}$ formula where $\Delta\Delta Ct = (C_t^{Telo} - C_t^{GAPDH})sample - (C_t^{Telo} - C_t^{GAPDH})reference$. The ICC(2,1) of the reactions for house sparrows is 0.88 in this lab, which is suggestive of high repeatability across plates.

Statistical analyses

Data selection

Three females failed to attend their nests in the incubation footage; these nests were removed from all analyses. For nests with multiple filmings, I chose to only include the one proximally associated with hatched nestlings, as otherwise it was not clear whether what was observed was true incubation behavior.

Defining variables of interest: Parental behavior models

I sought to determine whether males and females modified their incubation and postnatal behavior across with ambient temperature and date. Therefore, I chose to construct statistical models with the response variables of female incubation attentiveness, male incubation attentiveness, female postnatal attentiveness, male postnatal attentiveness, female postnatal visits and male postnatal visits. My explanatory variables of interest included film temperature (average temperature during the period of filming), date of filming (Julian day), and maximum clutch or brood size (clutch size for behavior observed during incubation and brood size for behavior observed during the postnatal period). Table A.6 summarizes and defines these variables of interest.

To calculate parental behavior, I used the BORIS time budget command to calculate frequency of visits and total duration of remain behavior for males and females in each video. To calculate shared behavior, I used the advanced event filtering command in BORIS to calculate the amount of time in which both the male and female were remaining. I then summed male and female remaining behavior and subtracted out the time in which the male and female were simultaneously present in the box.

Because the videos were different lengths, I calculated incubation and postnatal attentiveness as time remaining per hour by dividing time remaining by total run time of visible footage and then multiplied this number by 60. I calculated postnatal visits as visits per hour by dividing the number of times the bird entered the box by the total run of visible footage and then multiplied this number by 60. I completed these calculations for male behavior and female behavior. For film temperature, since all videos were filmed between 6am and 11am (when parental activity is likely to be highest), I averaged the hourly NOAA ambient temperature averages for that period for each day of filming.

Defining variables of interest: Nestling outcome models

I was also interested in understanding how ambient temperature, date and parental behavior shape both early and late developmental outcomes. Therefore, I chose to construct statistical models with the response variables of incubation period length, hatching survival, day 2 body mass (nestling mass at day 2 post-hatching, in grams) and day 2 telomere length (nestling relative telomere length obtained from a day 2 blood sample, in T/S ratio) for early developmental outcomes, and then day 10 body mass (nestling mass at day 10 post-hatching, in grams), day 10 telomere length (nestling relative telomere length obtained from a day 10 blood samples, in T/S ratio) and day 10 survival for late developmental outcomes. I calculated incubation period length

as the time between when the last egg was laid and the first nestling hatched. Hatching and day 10 survival were binary measures of whether or not an individual nestling hatched and survived to day 10, respectively, where failure = 0 and success = 1 (Table A.6).

My fixed explanatory variables of interest included average ambient temperatures during incubation and postnatal development. For average ambient temperature during incubation, I averaged the hourly values obtained from NOAA between the date of the last laid egg and first hatched nestling (incubation period length). For hatching survival, nests that completely failed obviously did not have a hatch date, so I took the average incubation period length for the dataset (10.61, rounded to 11), and assigned an "end" of incubation based on this average value. For average ambient temperature during postnatal growth, I averaged the NOAA hourly averages between the date of the first hatched nestling, and added ten days to that value for the "end" of the period, since body mass measures and blood samples for telomere length are taken around day 10 post-hatching, and the true day of day 10 is not known.

I was also interested in the explanatory variables of date of filming (Julian day), male and female incubation attentiveness, male and female postnatal attentiveness, as well as male and female postnatal visits. I also considered the effects of maximum clutch size (for early developmental outcomes) and maximum brood size (for late developmental outcomes), assay (for day 2 and day 10 telomere length models), and treatment effects (for late developmental outcomes), since all of these variables could also potentially explain variation in my response variables of interest.

Model building

All analyses were conducted in R version 4.0.3 (R Core Team, 2020). To avoid variance inflation, I conducted Pearson's Product-Moment Correlations between each of my explanatory

variables of interest, and opted not to include highly correlated variables (all with a $r > 0.50$) in the same model. The highest correlation between any of my variables included within the same model was $r = 0.45$ (Table A.7).

Average ambient temperature during incubation and average ambient temperature during postnatal growth exhibited a strong positive correlation ($r = 0.66$, $t = 5.51$, $df = 39$, $p < 0.0001$; Fig. 2.2). Due to this finding, and to avoid overfitting my models, I opted to build three separate models for each late developmental outcome (day 10 mass, day 10 telomere length and day 10 survival): one with average ambient temperature during incubation and incubation attentiveness, one with average ambient temperature during postnatal growth and postnatal attentiveness and one with average ambient temperature during postnatal growth and postnatal visits (Table A.8).

When I tested possible treatment effects on my late developmental outcomes, there was a significant treatment effect for day 10 mass and day 10 telomere length, but not day 10 survival (Table A.7). Therefore, treatment was dropped from the day 10 survival model.

Model selection

Date and ambient temperature exhibited a strong positive correlation ($r = 0.74$, $t = 7.58$, $df = 47$, $p < 0.0001$; Fig. 2.3), so I opted to build separate models for the ambient temperature and date effects for each response variable of interest (Table A.7), and in an attempt to parse out the effects of ambient temperature and the combination of ambient temperature and food availability (date) in shaping behavior and nestling outcomes. I then compared AIC values for each model, and selected one model over the other if the AIC value was less than two points lower. In some cases, the difference in AIC values was not sufficient to select one model, so both models were selected. See Table A.8 for a complete list of selected models.

Conducting the analysis

I utilized linear models for the response variables of incubation attentiveness, postnatal attentiveness, postnatal visits and incubation period length because there was only one measure per nest. For the response variables of day 2 body mass, day 2 telomere length, day 10 body mass and day 10 telomere length, I conducted linear mixed effect models (R 4.0.3 packages: nlme and MASS) and for hatching survival and day 10 survival, I used binomial generalized linear mixed effect models (R 4.0.3 packages: lme4, lmerTest and glmm). All explanatory variables were fit as fixed effects, and I also included nest as a random effect in these models because multiple individuals from the same nest were included in these analyses. Table A.8 summarizes the complete list of models used. I used the rsq package in R 4.0.3 to calculate model R^2 for the linear mixed effect and binomial generalized linear mixed effect models and used the adjusted R^2 for the linear models.

To determine differences in male and female behavior, I also conducted paired t tests for male and female incubation attentiveness, male and female postnatal attentiveness and male and female postnatal visits.

Figure 2.1. Female (left) and male (right) adult house sparrow (Peru Aves n.d.).

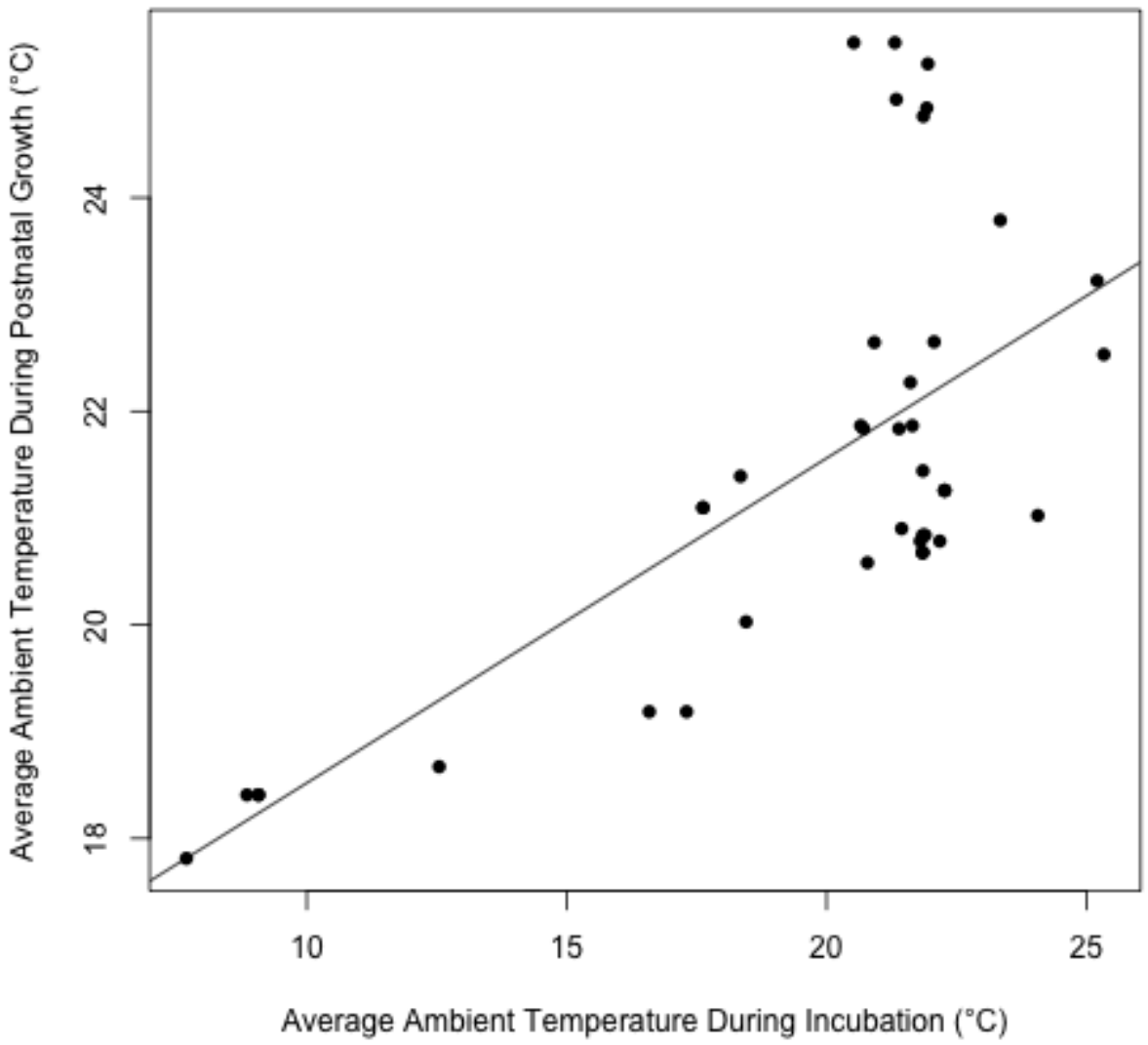

Figure 2.2. Average ambient temperature during postnatal growth plotted against average ambient temperature during incubation (n = 41).

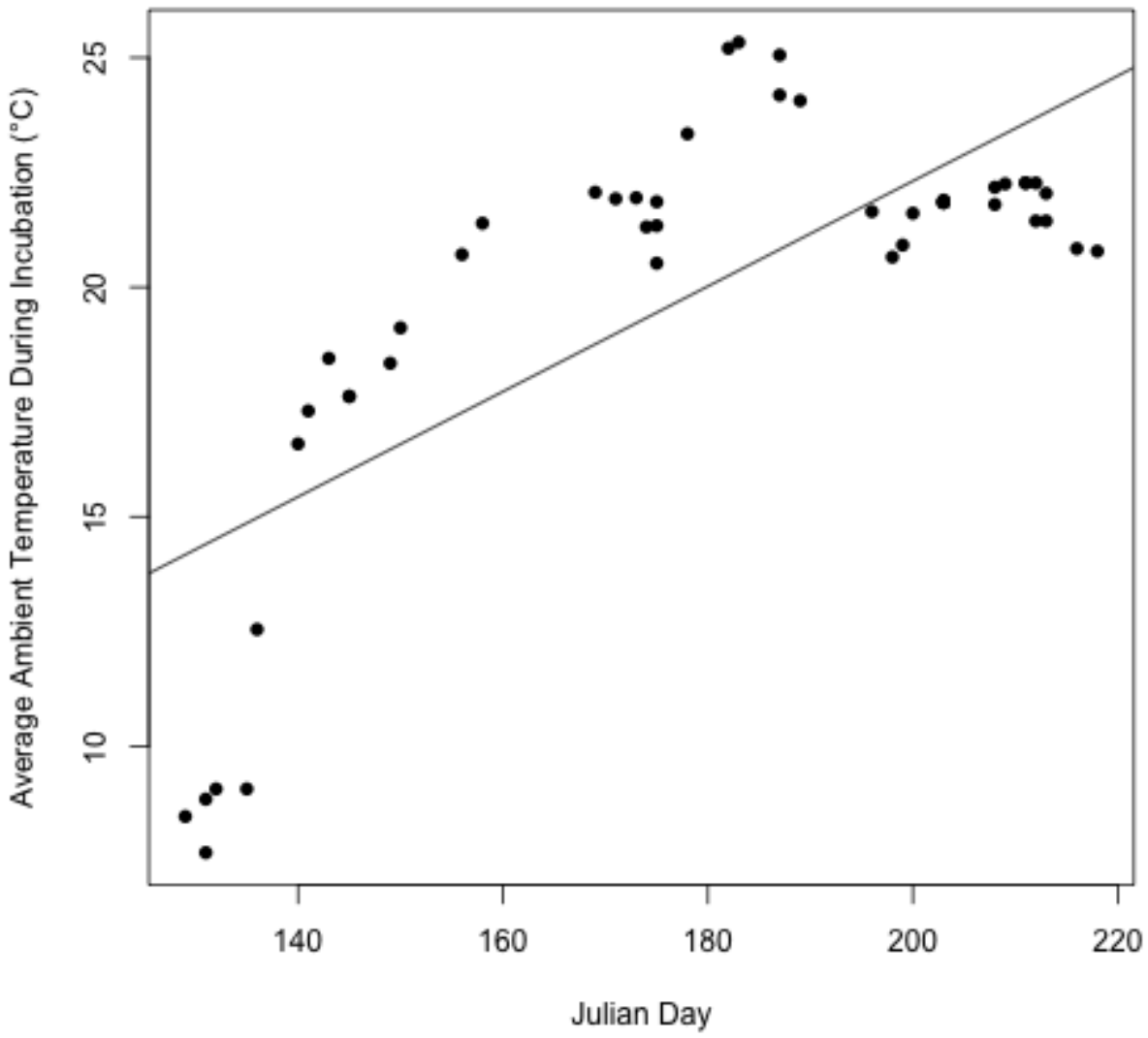

Figure 2.3. Average ambient temperature during incubation plotted against Julian day (n = 49).

Results

Effects of ambient temperature and date on parental behavior

Female incubation attentiveness decreased with average ambient temperature during filming (Estimate $\pm$ SE = - 0.64 $\pm$ 0.20, t = - 3.17, df = 53, p = 0.0025; Fig. 2.4) but not clutch size (Table 2.1). Male incubation attentiveness, female postnatal attentiveness, male postnatal attentiveness and female postnatal visits did not vary with any independent variables of interest

(Table 2.1). Male postnatal visits increased with brood size, but no other independent variables (Table 2.1).

Within a nest, females had higher incubation ($t = 11.45$, $df = 55$, $p < 0.0001$; Fig. 2.5) and postnatal ($t = 5.29$, $df = 40$, $p < 0.0001$; Fig. 2.6) attentiveness on average in comparison to males. Male and female house sparrows in this population exhibited a shared incubation attentiveness of 63%. There was no difference between the number of male and female visits within a nest ($t = 0.54$, $df = 40$, $p = 0.59$).

Effects of average ambient temperature during incubation and parental incubation attentiveness on early developmental outcomes

Incubation period length decreased with average ambient temperature during incubation (Estimate $\pm$ SE = - 0.11 $\pm$ 0.031, $t = - 3.78$, $df = 44$, $p = 0.00046$; Fig. 2.7) and clutch size (Table 2.1), but neither female nor male incubation attentiveness (Table 2.1). Hatching survival and day 2 body mass did not vary with explanatory variables of interest (Table 2.1). Day 2 telomere length decreased with average ambient temperature during incubation (Value $\pm$ SE = - 0.048 $\pm$ 0.014, $t = - 3.30$, $df = 27$, $p = 0.0027$; Fig. 2.8) and varied with assay but not any other independent variables (Table 2.1).

Effects of average ambient temperature and parental behavior during incubation and postnatal growth on late developmental outcomes

Average ambient temperature during incubation but not average ambient temperature during postnatal growth positively predicted day 10 mass (Value $\pm$ SE = 0.18 $\pm$ 0.088, $t = 2.09$, $df = 35$, $p = 0.043$; Fig. 2.9). Day 10 body mass also varied with treatment but none of the other variables of interest (Table 2.1). Other than assay and treatment in some selected models, no independent variables of interest predicted day 10 telomere length (Table 2.1). Number of female

visits during postnatal growth (Table 2.1; Fig. 2.10) and brood size (in some selected models; see Table 2.1) positively predicted day 10 survival, but day 10 survival did not vary with any other explanatory variables of interest (Table 2.1).

Table 2.1. Results from linear models for response variables of interest.

Response Variable	Effects	Estimate	SE	t value	DF	p value
Female incubation	(Intercept)	30.05	7.11	4.34	53	< 0.0001 *
attentiveness	Average ambient temperature during incubation	-0.64	0.20	- 3.17	53	**0.0025 ***
Adj. R^2 = 0.13	Clutch size	2.06	1.38	1.49	53	0.14
Male incubation	(Intercept)	12.20	6.62	1.81	53	0.075
attentiveness	Average ambient temperature during incubation	0.00017	0.19	0.001	53	0.99
Adj. R^2 = -0.035	Clutch size	- 0.39	1.29	-0.30	53	0.76
Male incubation	(Intercept)	5.74	8.66	0.66	53	0.51
attentiveness	Date	0.035	0.033	1.04	53	0.30
Adj. R^2 = -0.014	Clutch size	- 0.43	1.24	-0.35	53	0.73
Female postnatal	(Intercept)	25.90	9.76	2.65	38	0.011 *
attentiveness	Average ambient temperature during filming	-0.78	0.42	-1.86	38	0.069
Adj. R^2 = 0.054	Brood size	1.37	1.12	1.22	38	0.22
Female postnatal	(Intercept)	-6.64	10.13	-0.65	38	0.51
attentiveness	Date	0.089	0.048	1.84	38	0.072
Adj. R^2 = 0.052	Brood size	0.94	1.10	0.85	38	0.39
Female postnatal	(Intercept)	7.63	5.99	1.27	38	0.21
visits	Average ambient temperature during filming	-0.14	0.25	-0.57	38	0.57
Adj. R^2 = 0.013	Brood size	1.08	0.69	1.57	38	0.12
Female postnatal	(Intercept)	11.81	6.10	1.93	38	0.060
visits	Date	-0.038	0.029	-1.33	38	0.19
	Brood size	1.03	0.66	1.54	38	0.13
Adj. R^2 = 0.049						
Male postnatal	(Intercept)	2.26	6.87	0.32	38	0.74
attentiveness	Average ambient temperature during filming	0.15	0.29	0.52	38	0.60
Adj. R^2 = -0.044	Brood size	-0.097	0.79	-0.12	38	0.90
Male postnatal	(Intercept)	-2.75	6.98	-0.39	38	0.69
attentiveness	Date	0.044	0.033	1.324	38	0.19
	Brood size	-0.043	0.76	-0.057	38	0.95
Adj. R^2 = -0.0061						
Male postnatal	(Intercept)	-6.24	6.53	-0.95	38	0.34
visits	Average ambient temperature during filming	0.35	0.28	1.25	38	0.21
Adj. R^2 = 0.11	Brood size	1.58	0.75	2.10	38	**0.041 ***
Male postnatal	(Intercept)	10.67	6.66	1.6	38	0.11

Table 2.1. Results from linear models for response variables of interest (continued).

Response Variable	Effects	Estimate	SE	t value	DF	p value
visits	Date	-0.052	0.031	-1.65	38	0.10
Adj. R^2 = 0.14	Brood size	1.78	0.73	2.44	38	**0.019 ***
Incubation period length	(Intercept)	15.79	1.19	13.25	44	< 0.0001 *
	Female incubation attentiveness	-0.029	0.020	-1.43	44	0.15
	Male incubation attentiveness	-0.024	0.019	-1.28	44	0.20
Adj. R^2 = 0.30	Average ambient temperature during incubation	-0.11	0.031	-3.78	44	**0.00046 ***
	Clutch size	-0.33	0.16	-2.05	44	**0.046 ***
Response Variable	Effect	Estimate	SE	z value	DF.r$^+$	p value
Hatching survival	(Intercept)	-0.69	1.84	-0.37	238	0.70
	Female incubation attentiveness	0.040	0.029	1.35	238	0.17
Model R^2 = 0.18	Male incubation attentiveness	-0.0080	0.029	-0.27	238	0.78
	Average ambient temperature during incubation	0.024	0.050	0.47	238	0.63
	Clutch size	0.093	0.24	0.37	238	0.70
Hatching survival	(Intercept)	1.40	0.22	6.22	238	< 0.0001 *
	Female incubation attentiveness	0.29	0.20	1.44	238	0.14
Model R^2 = 0.18	Male incubation attentiveness	-0.079	0.20	-0.38	238	0.69
	Date	0.18	0.20	0.90	238	0.36
	Clutch size	0.093	0.19	0.48	238	0.63
Response Variable	Effect	Value	SE	t value	DF	p value
Day 2 body mass	(Intercept)	8.84	2.39	3.68	92	0.0004 *
	Female incubation attentiveness	-0.025	0.041	-0.60	27	0.54
Model R^2 = 0.54	Male incubation attentiveness	-0.066	0.046	-1.42	27	0.16
	Average ambient temperature during incubation	0.10	0.064	1.55	27	0.13
	Clutch size	-0.66	0.35	-1.89	27	0.068
Day 2 telomere length	(Intercept)	1.42	0.49	2.88	73	0.0052 *
	Female incubation attentiveness	0.0080	0.0083	0.97	27	0.34
	Male incubation attentiveness	-0.012	0.0090	-1.35	27	0.18
Model R^2 = 0.68	Average ambient temperature during incubation	-0.048	0.014	-3.30	27	**0.0027 ***
	Clutch size	0.041	0.067	0.62	27	0.54
	Assay 2	-0.51	0.40	-1.30	73	0.19
	Assay 3	-0.081	0.33	-0.24	73	0.80
	Assay 4	0.25	0.28	0.90	73	0.36
	Assay 5	0.23	0.30	0.75	73	0.45
	Assay 6	-0.23	0.28	-0.83	73	0.41
	Assay 7	0.018	0.27	0.067	73	0.94

Table 2.1. Results from linear models for response variables of interest (continued).

Response Variable	Effect	Value	SE	t value	DF	p value
	Assay 8	0.33	0.26	1.23	73	0.22
	Assay 9	0.22	0.27	0.83	73	0.40
	Assay 10	0.20	0.28	0.71	73	0.47
	Assay 11	0.46	0.28	1.64	73	0.10
	Assay 12	0.12	0.27	0.44	73	0.66
	Assay 13	-0.097	0.27	-0.36	73	0.72
	Assay 14	0.32	0.29	1.10	73	0.27
	Assay 18	0.067	0.32	0.21	73	0.83
	Assay 19	0.66	0.32	2.02	73	**0.047 ***
	Assay 20	-0.13	0.33	-0.42	73	0.67
	Assay 21	0.24	0.32	0.75	73	0.45
	Assay 22	0.14	0.28	0.51	73	0.61
Day 10 body mass	(Intercept)	21.98	3.00	7.31	109	< 0.0001 *
	Female incubation attentiveness	-0.021	0.055	-0.39	35	0.69
Model R^2 = 0.55	Male incubation attentiveness	0.0063	0.052	0.12	35	0.90
	Average ambient temperature during incubation	0.18	0.088	2.09	35	**0.043 ***
	Treatment: natural variation	2.20	0.77	2.84	109	**0.0053 ***
	Treatment: stress	-0.58	0.46	-1.26	109	0.20
	Brood size	-0.29	0.31	-0.93	35	0.35
Day 10 body mass	(Intercept)	20.08	5.19	3.86	105	0.0002*
	Female postnatal attentiveness	0.014	0.045	0.32	33	0.74
Model R^2 = 0.54	Male postnatal attentiveness	0.061	0.073	0.83	33	0.41
	Average ambient temperature during postnatal growth	0.17	0.21	0.79	33	0.43
	Treatment: natural variation	2.81	0.80	3.49	105	**0.0007 ***
	Treatment: stress	-0.58	0.47	-1.22	105	0.22
	Brood size	-0.15	0.33	-0.46	33	0.64
Day 10 body mass	(Intercept)	18.38	5.02	3.65	105	0.0004 *
	Female postnatal visits	0.002	0.078	0.027	33	0.97
Model R^2 = 0.54	Male postnatal visits	-0.090	0.062	-1.44	33	0.15
	Average ambient temperature during postnatal growth	0.27	0.19	1.39	33	0.17
	Treatment: natural variation	2.87	0.77	3.70	105	**0.0003 ***
	Treatment: stress	-0.54	0.47	-1.15	105	0.25
	Brood size	0.0057	0.34	0.016	33	0.98
Day 10 telomere length	(Intercept)	1.04	0.39	2.65	84	0.0097 *
	Female incubation attentiveness	-0.0093	0.0068	-1.38	34	0.17
	Male incubation attentiveness	-0.0051	0.0063	-0.82	34	0.41

Table 2.1. Results from linear models for response variables of interest (continued).

Response Variable	Effect	Value	SE	t value	DF	p value
Model $R^2 = 0.58$	Average ambient temperature during incubation	0.0013	0.012	0.10	34	0.91
	Treatment: natural variation	0.060	0.18	0.32	84	0.74
	Treatment: stress	0.10	0.054	1.94	84	0.055
	Brood size	-0.0058	0.037	-0.16	34	0.87
	Assay 2	-0.44	0.29	-1.52	84	0.13
	Assay 3	-0.024	0.22	-0.11	84	0.91
	Assay 4	0.76	0.27	2.83	84	**0.0058 ***
	Assay 5	0.14	0.25	0.56	84	0.57
	Assay 6	0.25	0.25	0.99	84	0.32
	Assay 7	0.23	0.24	0.96	84	0.33
	Assay 8	0.53	0.24	2.21	84	**0.030 ***
	Assay 9	0.089	0.27	0.33	84	0.74
	Assay 10	0.12	0.23	0.53	84	0.59
	Assay 11	0.76	0.28	2.72	84	**0.0079 ***
	Assay 12	0.094	0.25	0.37	84	0.71
	Assay 13	0.035	0.24	0.14	84	0.88
	Assay 14	0.13	0.36	0.37	84	0.71
	Assay 15	-0.10	0.33	-0.30	84	0.76
	Assay 16	0.097	0.38	0.25	84	0.79
	Assay 17	0.51	0.31	1.65	84	0.10
	Assay 18	0.43	0.37	1.15	84	0.25
	Assay 19	0.74	0.37	1.97	84	0.052
	Assay 20	0.49	0.28	1.75	84	0.083
	Assay 21	0.60	0.28	2.13	84	**0.036 ***
	Assay 22	0.39	0.28	1.40	84	0.16
Day 10 telomere length	(Intercept)	0.43	0.78	0.55	82	0.58
	Female postnatal attentiveness	-0.0096	0.0057	-1.66	33	0.10
	Male postnatal attentiveness	-0.0060	0.0084	-0.71	33	0.47
Model $R^2 = 0.58$	Average ambient temperature during postnatal growth	0.021	0.0321	0.67	33	0.50
	Treatment: natural variation	0.065	0.159	0.41	82	0.68
	Treatment: stress	0.10	0.054	1.97	82	0.051
	Brood size	-0.0048	0.037	-0.12	33	0.89
	Assay 2	-0.47	0.27	-1.70	82	0.091
	Assay 3	0.046	0.22	0.20	82	0.84
	Assay 4	0.73	0.25	2.84	82	**0.0056 ***
	Assay 5	0.26	0.27	0.98	82	0.32
	Assay 6	0.17	0.23	0.74	82	0.46
	Assay 7	0.22	0.24	0.92	82	0.35

Table 2.1. Results from linear models for response variables of interest (continued).

Response Variable	Effect	Value	SE	t value	DF	p value
	Assay 8	0.43	0.23	1.84	82	0.069
	Assay 9	-0.034	0.27	-0.12	82	0.90
	Assay 10	0.15	0.24	0.62	82	0.53
	Assay 11	0.73	0.26	2.71	82	**0.0080 ***
	Assay 12	0.023	0.26	0.089	82	0.92
	Assay 13	0.042	0.23	0.17	82	0.85
	Assay 14	0.072	0.36	0.19	82	0.84
	Assay 15	-0.0013	0.33	-0.0040	82	0.99
	Assay 16	0.056	0.36	0.15	82	0.87
	Assay 17	0.60	0.30	2.00	82	**0.048 ***
	Assay 18	0.51	0.36	1.40	82	0.16
	Assay 19	0.81	0.36	2.25	82	**0.026 ***
	Assay 20	0.53	0.26	2.00	82	**0.048 ***
	Assay 21	0.63	0.26	2.37	82	**0.020 ***
	Assay 22	0.44	0.26	1.67	82	0.097
Day 10 telomere length Model $R^2 = 0.58$	(Intercept)	0.53	0.80	0.66	82	0.50
	Female postnatal visits	-0.0067	0.0089	-0.75	33	0.45
	Male postnatal visits	-0.0020	0.0078	-0.25	33	0.79
	Average ambient temperature during postnatal growth	0.015	0.031	0.51	33	0.61
	Treatment: natural variation	0.027	0.16	0.16	82	0.86
	Treatment: stress	0.10	0.055	1.95	82	0.054
	Brood size	0.0057	0.040	0.14	33	0.88
	Assay 2	-0.50	0.28	-1.76	82	0.080
	Assay 3	-0.049	0.23	-0.21	82	0.83
	Assay 4	0.69	0.26	2.67	82	**0.0090 ***
	Assay 5	0.042	0.25	0.16	82	0.86
	Assay 6	0.10	0.24	0.43	82	0.66
	Assay 7	0.16	0.25	0.64	82	0.52
	Assay 8	0.42	0.25	1.68	82	0.095
	Assay 9	-0.019	0.27	-0.070	82	0.94
	Assay 10	0.021	0.24	0.087	82	0.93
	Assay 11	0.61	0.28	2.18	82	**0.031 ***
	Assay 12	-0.075	0.26	-0.28	82	0.77
	Assay 13	-0.10	0.24	-0.41	82	0.68
	Assay 14	-0.065	0.35	-0.18	82	0.85
	Assay 15	-0.12	0.33	-0.38	82	0.70
	Assay 16	0.11	0.39	0.27	82	0.78
	Assay 17	0.45	0.30	1.48	82	0.14

Table 2.1. Results from linear models for response variables of interest (continued).

Response Variable	Effect	Value	SE	t value	DF	p value
	Assay 18	0.36	0.36	0.99	82	0.32
	Assay 19	0.67	0.36	1.83	82	0.069
	Assay 20	0.42	0.27	1.56	82	0.12
	Assay 21	0.51	0.27	1.87	82	0.064
	Assay 22	0.31	0.26	1.16	82	0.24
Response Variable	Effect	Estimate	SE	z value	DF.r[+]	p value
Day 10 survival	(Intercept)	-1.51	3.38	-0.44	173	0.65
	Female incubation attentiveness	-0.0034	0.065	-0.053	173	0.95
Model R^2 = 0.48	Male incubation attentiveness	-0.010	0.060	-0.17	173	0.86
	Average ambient temperature during incubation	0.044	0.094	0.46	173	0.64
	Brood size	0.82	0.36	2.23	173	**0.025 ***
Day 10 survival	(Intercept)	2.69	0.59	4.54	173	< 0.0001 *
	Male incubation attentiveness	-0.090	0.41	-0.21	173	0.82
Model R^2 = 0.47	Female incubation attentiveness	0.035	0.42	0.084	173	0.93
	Date	0.52	0.41	1.25	173	0.21
	Brood size	0.89	0.39	2.27	173	**0.023 ***
Day 10 survival	(Intercept)	2.84	0.59	4.78	161	< 0.0001 *
	Female postnatal attentiveness	0.58	0.41	1.40	161	0.15
Model R^2 = 0.32	Male postnatal attentiveness	-0.23	0.38	-0.61	161	0.54
	Average ambient temperature during postnatal growth	0.022	0.40	0.054	161	0.95
	Brood size	0.052	0.37	0.13	161	0.89
Day 10 survival	(Intercept)	2.83	0.58	4.88	161	< 0.0001 *
	Female postnatal attentiveness	0.51	0.39	1.31	161	0.19
Model R^2 = 0.31	Male postnatal attentiveness	-0.31	0.36	-0.88	161	0.37
	Date	0.33	0.42	0.80	161	0.42
	Brood size	0.044	0.37	0.11	161	0.90
Day 10 survival	(Intercept)	2.78	0.52	5.28	161	< 0.0001 *
	Female postnatal visits	1.28	0.49	2.61	161	**0.0090 ***
Model R^2 = 0.27	Male postnatal visits	0.043	0.35	0.12	161	0.90
	Average ambient temperature during postnatal growth	-0.019	0.34	-0.058	161	0.95
	Brood size	-0.27	0.39	-0.70	161	0.48
Day 10 survival	(Intercept)	2.80	0.53	5.27	161	< 0.0001 *
	Female postnatal visits	1.25	0.48	2.61	161	**0.0089 ***
Model R^2 = 0.28	Male postnatal visits	0.085	0.32	0.26	161	0.79
	Date	0.37	0.37	1.01	161	0.30
	Brood size	-0.24	0.39	-0.62	161	0.53

[+] DF.r = residual degrees of freedom

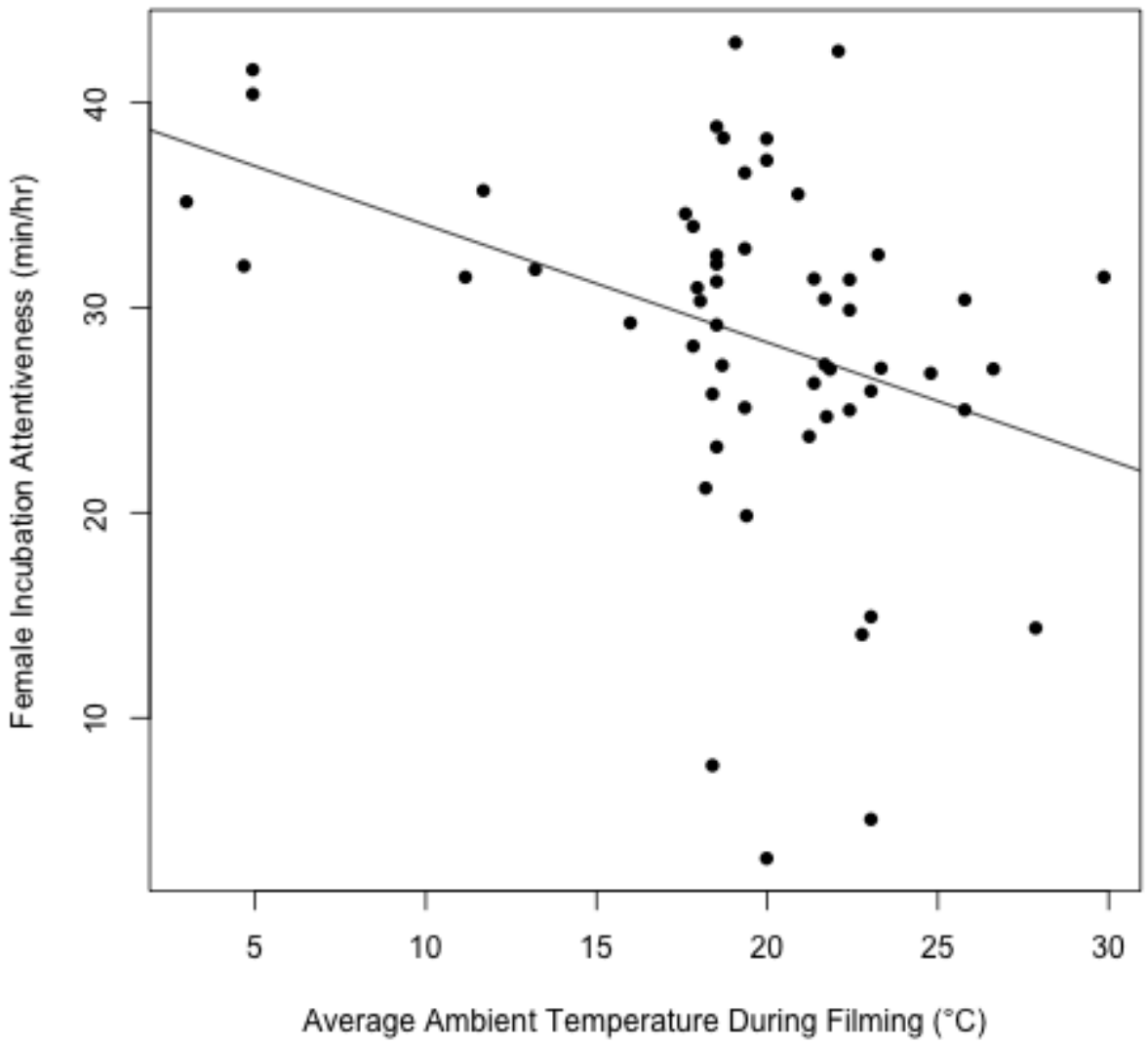

Figure 2.4. Female incubation attentiveness plotted against average temperature during filming (n = 56)

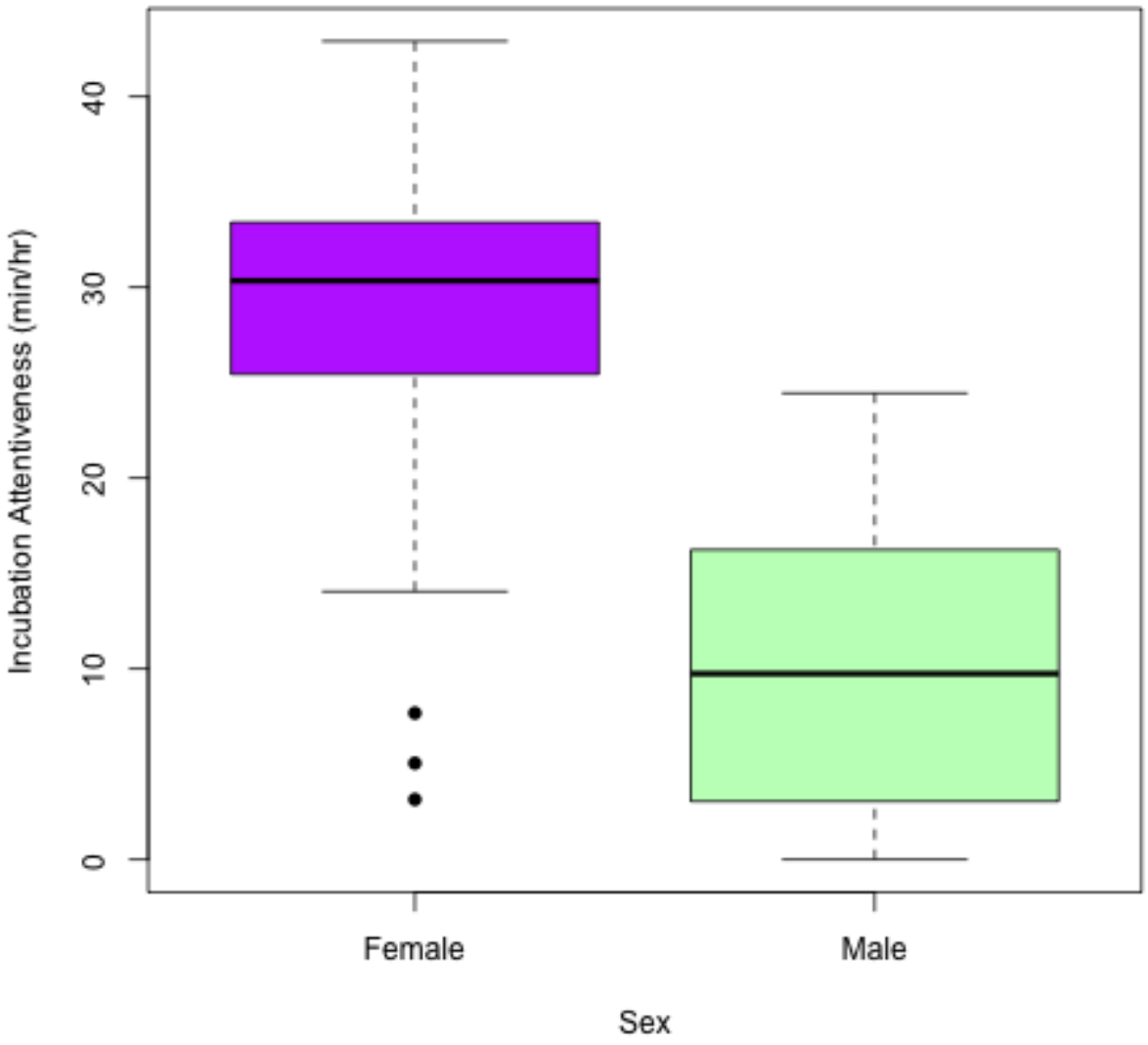

Figure 2.5. Boxplot depicting female and male incubation attentiveness (n = 56).

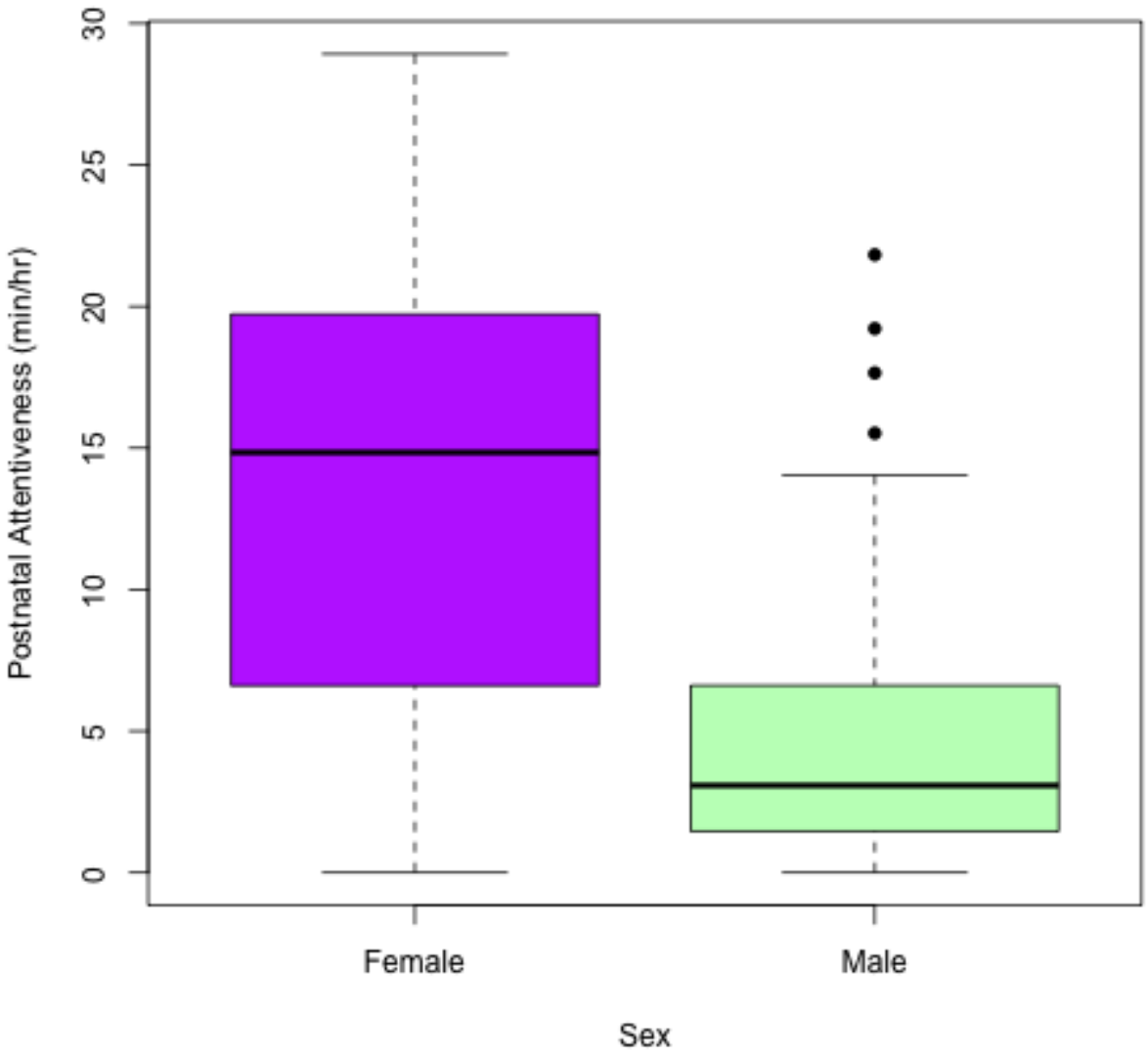

Figure 2.6. Boxplot depicting female and male postnatal attentiveness (n = 41).

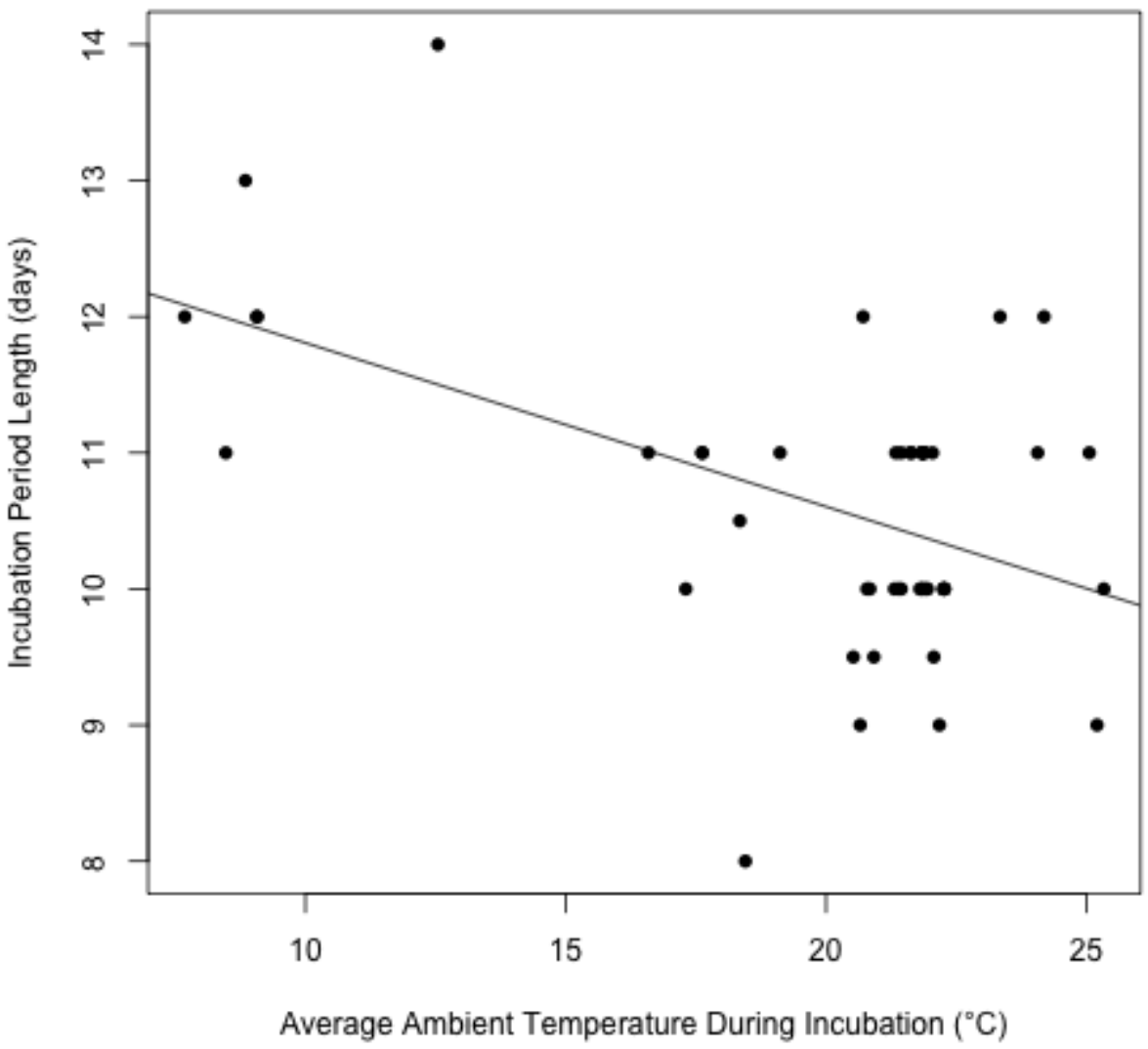

Figure 2.7. Incubation period length plotted against average ambient temperature during incubation (n = 49).

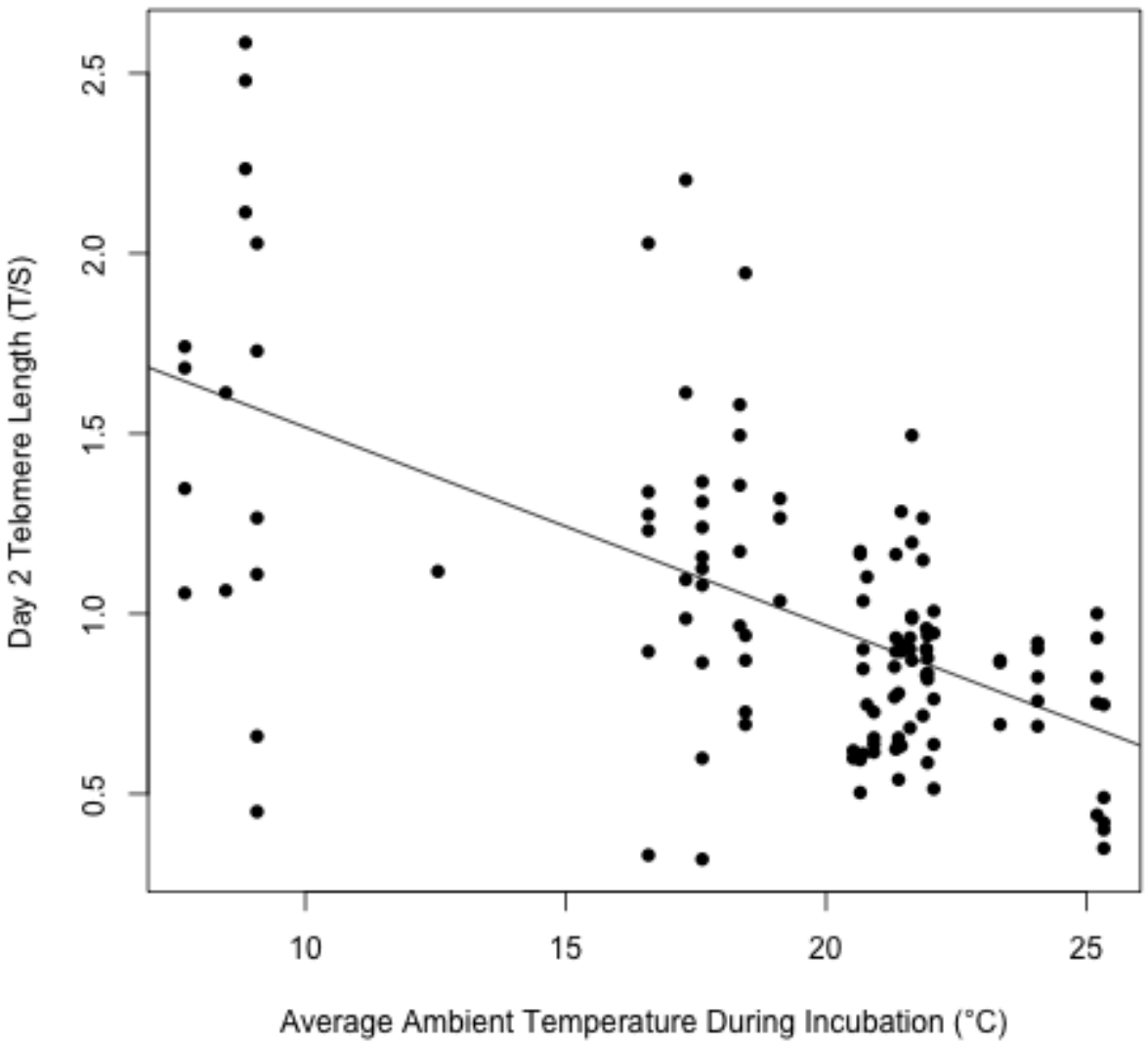

Figure 2.8. Day 2 telomere length plotted against average ambient temperature during incubation (n = 123 birds from n = 32 nests).

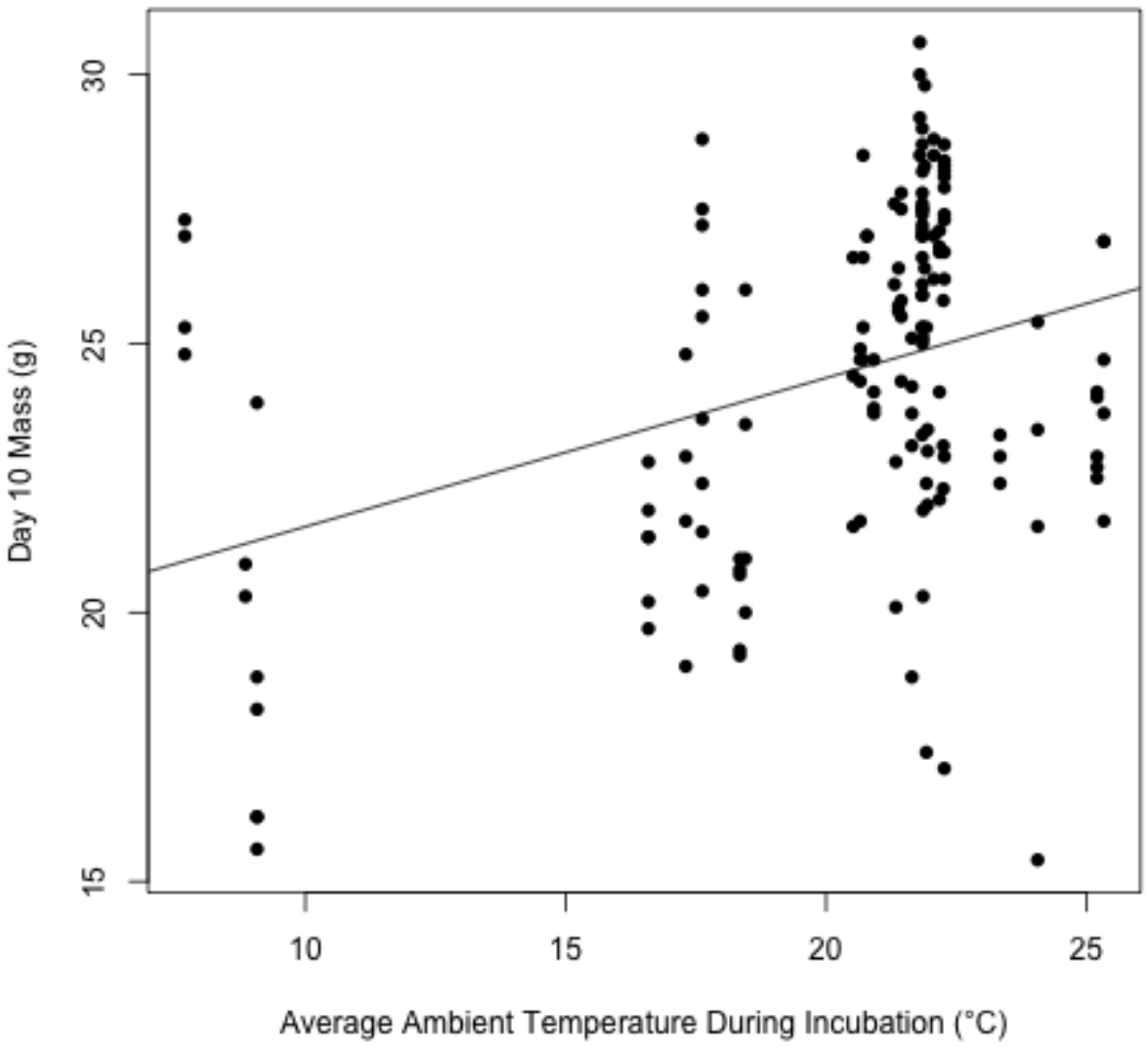

Figure 2.9. Day 10 mass plotted against average ambient temperature during incubation (n = 151 birds from n = 40 nests).

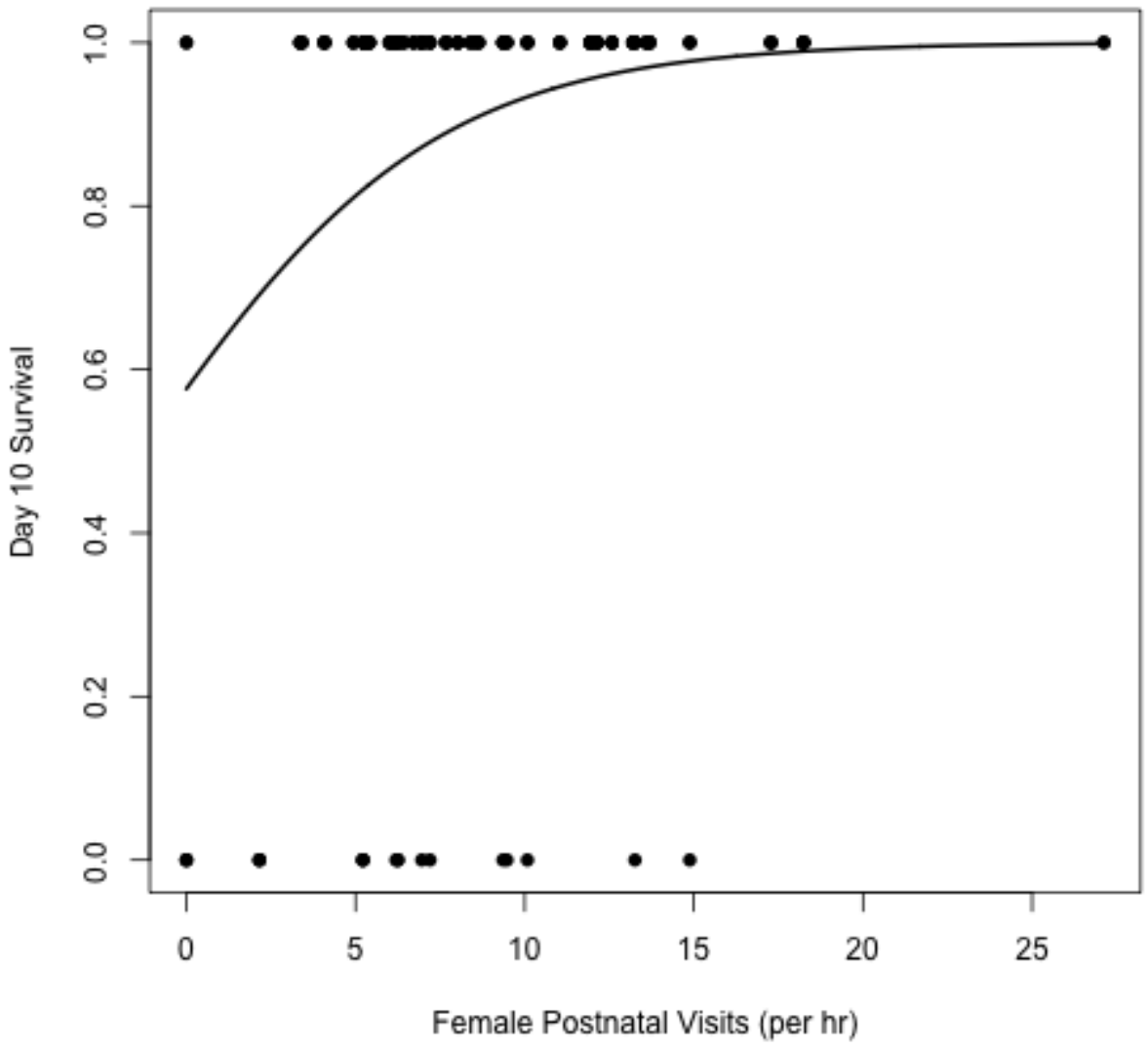

Figure 2.10. Day 10 survival plotted against female postnatal visits (n = 167 birds from n = 41 nests).

Discussion

This study sought to determine the contributions of ambient temperature and parental behavior on developmental outcomes across incubation and postnatal growth in an altricial species. To the best of my knowledge, this has never been done before in a single study. In sum, these results indicate that females but not males modify their behavior in response to temperature, average ambient temperature during incubation predicts early developmental outcomes better than

parental behavior does, female behavior predicts some late developmental outcomes (day 10 survival), and body mass effects of average ambient temperature during incubation can carry over to late postnatal growth. The effects of parental behavior and ambient temperature across ontogeny appears to be context-dependent.

Females but not males modify their behavior in response to average ambient temperature during filming

Maintaining a consistent thermal environment during early stages of development prior to the transition to endothermy is a crucial determinant of fitness outcomes. Therefore, it is not surprising that females decreased incubation attentiveness with average ambient temperature during filming. This same pattern of decreased attentiveness with temperature is reflected in yellow warblers (Rohwer & Purcell, 2019), and female eiders with experimentally decreased down in their nests (causing nests to cool faster) also increased attentiveness (McClintock et al., 2014). Under warmer conditions, eggs cool less during off-bouts (Conway & Martin, 2000) and it is less important for the female to exert herself rewarming them or preventing them from cooling (Vleck, 1981). This also allows the female to use this time to forage or take part in other forms of self-maintenance (Álvarez & Barba, 2014).

My finding that females incubate more than males is supported by the literature, and further, females have warmer abdomens (Bartlett et al., 2005). It may be more important for the female to fine-tune her behavior with temperature to tightly regulate the nest environment. Males did not modify their incubation attentiveness with date or temperature, but since their contribution is lower and less effective in comparison to females, males may not need to fine-tune their incubation behavior with abiotic conditions to regulate the developmental environment.

Postnatal attentiveness did not vary with average ambient temperature during postnatal growth or date, although females did spend more time in the nest in comparison to males. This may be related with the stage of development during which the videos were filmed. Filming occurred around day 6 post-hatching, after when altricial birds begin to transition from ectothermy to endothermy (Andrew et al., 2017). While nestlings may not be able to thermoregulate completely on their own, they may not rely as much on parental brooding as they would at earlier stages (Yoon et al., 2016). If these videos were filmed earlier in the development, more of a temperature effect on brooding behavior may have been observed (Yoon et al., 2016), and also more of an effect of postnatal attentiveness on late developmental outcomes. Feeding rate also did not vary with average ambient temperature during postnatal growth or date, although male postnatal visits increased with brood size. This is not surprising given that a larger brood would likely require more food. While parents did not appear to shift how much they fed with temperature and date, they may have shifted what they fed their young, as abundance for different prey types changes seasonally for house sparrows (Anderson 2006).

Average ambient temperature during incubation predicts early developmental outcomes better than parental behavior

Warm incubation temperatures typically promote faster embryonic growth (Ospina et al., 2018; Stier et al., 2020), which would explain why these birds exhibited shorter incubation period lengths. Higher temperatures would also promote more rapid cell divisions (and lower temperatures would decrease the rate of cell divisions), which might also explain why day 2 telomere length decreased with average ambient temperature during incubation (Stier et al., 2020; Vedder et al., 2018).

Even with a faster growth rate during incubation, there are mixed results for the effects of temperature on early nestling size. Some studies found that cold-incubated birds were smaller at hatching (Nord & Nilsson, 2021; Vedder et al., 2018) and heated chicks were heavier at hatching (Carvalho et al., 2020). However, other studies found that body mass at hatching or during early postnatal growth did not vary with temperature conditions experienced during incubation (Wada et al., 2015; Stier et al., 2020). It is possible that not enough variation in day 2 body mass exists to reflect any effects from the explanatory variables of interest in this study. In the literature, prolonged exposure to extreme incubation temperatures can attenuate hatching success (Wada et al., 2015; Stier et al., 2020; Carvalho et al., 2020). However, it is entirely possible that birds at the Fargo field site never experienced developmental temperatures extreme enough to attenuate hatching survival.

Given that females modify incubation behavior with ambient temperature, it was surprising that female incubation attentiveness was not a better predictor of early developmental outcomes. It is possible that the birds have chosen nest sites (Carroll et al., 2018) and built their nests in such a way that heat is well-retained (Heenan, 2013), and eggs and nestlings did not experience a great change in temperature during parental off-bouts. In cavity nesting birds in temperate climates, nest size and thickness can be especially important in shaping hatching and fledging success (Akresh et al., 2017). Variation in nest size and thickness exists at the Fargo field site, so future studies could examine these effects, perhaps utilizing iButtons to quantify them.

It is also worth considering that altricial birds generally experience more variation in temperature during development than precocial birds do (Ospina et al., 2018). For instance, female wood ducks generally incubate more than 80% of day (Hepp et al., 2006), while according to my shared incubation calculation for this field site, house sparrow parents combined incubate an

average of about 63% of the time in the videos, meaning that the eggs would be unattended for 37% of the time. Further, birds at the Fargo field site experience high thermal heterogeneity across the calendar year (Dennis, 2022 [unpublished]). It is possible that house sparrows in this population are resilient to variability in temperature during early development, so parental incubation behavior may be less important in shaping developmental outcomes.

Female behavior predicts some late developmental outcomes

Female postnatal visits positively predicted day 10 survival. This is not surprising, as larger fledglings typically have greater fitness (Peach et al., 2015). Further, better fed late-stage nestlings have more energy to thermoregulate (Yoon et al., 2016), and since at this point, the transition from ectothermy to endothermy has begun, this may partially explain why day 6 feeding behavior explains day 10 survival better than either of the buffering behaviors (incubation and postnatal attentiveness) or average ambient temperature during incubation and postnatal development.

There was no difference in male and female feeding rates, which in itself is not entirely surprising. Male and female feeding rates can vary with populations, and in some orange-crowned warbler populations, there is no difference in male and female feeding rates (Yoon et al., 2016). However, considering that there was no difference in male and female feeding rate, it was not clear why male behavior would not have influenced day 10 survival as well. It is possible that females are somehow more effective feeders, and they bring more nutrient-rich food that provides the nestlings more energy to grow, become large fledglings and successfully thermoregulate as they transition to endothermy. There is evidence in thorn-tailed rayaditos (a biparental species) that females typically provide a greater proportion of insect larvae in comparison to the father, whose prey items are more varied (Espíndola-Hernández et al., 2017).

Effects of average ambient temperature during incubation can carry over to late postnatal growth

In this population, day 10 body mass increased with average ambient temperature during incubation, indicating that faster growth during incubation promoted accumulation of body mass. Ospina et al. (2018) also found body mass differences with incubation temperature in late postnatal development that were not apparent at hatching. Because average ambient temperature during postnatal growth did not predict day 10 body mass in the Fargo population, the variation observed in day 10 body mass is most likely a carryover effect of incubation conditions rather than a result of temperature conditions experienced during postnatal growth. The effect of average ambient temperature during incubation on telomeres evident at day 2 did not carry over to day 10, however. The most likely explanation for this was that multiple measures existed for very few birds in this dataset. Some birds were not sampled at day 2, and some birds sampled at day 2 perished before a day 10 sample could be obtained. To ascertain this, an analysis of change in telomere length analysis should be completed in the future with a larger sample size.

Implications of these findings and future directions

The results of this study indicate that the effects of parental behavior and ambient temperature across ontogeny are context-dependent; sometimes parental behavior predicts developmental outcomes, often ambient temperature is a better predictor, and the effects of early conditions can persist to later stages of development. While this study addresses gaps in the literature, it also highlights new questions to answer. If females modify parental behavior with temperature, why does incubation attentiveness not better predict early developmental outcomes, and why do females continue to invest time and energy in incubation? Further, considering that no male behavior predicted any developmental outcomes, why does selection favor male attentiveness

and feeding behavior in this population (if in fact it does)? Future studies should seek to answer these questions.

CHAPTER 4. CONCLUSIONS AND IMPLICATIONS

In this thesis, I sought to determine the relative contributions of seasonal thermal profile and variance in ambient and nest temperature during incubation, as well as the influence of parental behavior and ambient temperature across ontogeny in shaping early- and late-stage growth, telomere and survival outcomes in house sparrow nestlings. I found that the effects of each of these factors was context-dependent; certain outcomes were impacted differently across developmental stages. In the chapter 2, seasonal thermal profile of temperature was important in shaping growth rate during incubation and body mass measures across ontogeny. However, consistency of temperature was also important in shaping incubation period length, as well as survival to hatching. In chapter 3, averages in temperature dictated incubation period length, day 2 telomere length and day 10 body mass, and female behavior shaped day 10 telomere length and survival to day 10.

With such mixed findings, it is difficult to paint a clear picture of fitness implications of traits associated with variation in thermal seasonal thermal profile and consistency and parental behavior, or how to create optimal developmental conditions. However, perhaps this is also context-dependent. For instance, short telomeres at 25 days post-hatching are linked to decreased longevity in zebra finches (Heidinger et al., 2012), but large house sparrow fledglings are more likely to survive to adulthood and reproduce (Peach et al., 2015). A bird could develop quickly during the postnatal period and exhibit short telomeres, but accumulate a great deal of body mass and successfully fledge. This bird could still potentially have high fitness if it produced many offspring before its short lifespan came to an end. Similarly, a bird might develop slowly during incubation and hatch with long telomeres, but if it has large amount of residual yolk and is too small to hatch from the egg (Rubin et al., 2021), then these longer telomeres would not benefit this bird in the long-run. Or, it is also possible that birds with intermediate phenotypes would have the

highest fitness. The only way to determine any of this would be to attempt to recapture these birds as adults, which future studies should seek to do.

Whether or not the traits of interest are truly predictive of fitness may also be context-dependent in terms of interactions between developmental environments across ontogeny for an individual. Several theories exist for how interacting environmental conditions shape late-stage phenotypes. For instance, the silver spoon hypothesis states that individuals who experience beneficial conditions during early and/or adulthood will confer a fitness advantage over others experiencing poor conditions at any point in development (Butler & McGraw, 2012). During development, embryos receive cues indicating the environmental conditions they will likely experience after partuition, and organisms modify development in response to these cues (Monaghan, 2008). However, this can be problematic when developmental environment does not match the adult environment, as there can be steep longitudinal consequences. This is known as the environmental matching hypothesis (Butler & McGraw, 2012), but similar to the silver spoon hypothesis, most of the work has centered around biomedical studies and/or nutrient availability. With increasingly erratic global temperatures (Thornton et al., 2014), environmental mismatch could potentially become increasingly prevalent during development. Future studies should seek to test the silver spoon and environmental matching hypotheses in the context of developmental temperature and parental behavior in order to determine how resilient individuals are to unreliable environmental cues.

Last, different species and populations could potentially be impacted more or less adversely by extreme and erratic temperatures and unfavorable parental behavior. The house sparrows at the Fargo field site experience a great deal of thermal heterogeneity across the calendar year, and parents who are off the nest nearly 40% of the time, in contrast to wood duck females and parents

from other precocial species who are off the nest less than 20% of the time during the day (Hepp et al., 2006). Because these are conditions they typically experience, they may be more resilient to inconsistency and thermal extremes than other species or even other house sparrow populations across a latitudinal gradient. Thermal environment, parental behavior and developmental outcomes can vary across species and populations. For instance, tropical birds tend to exhibit less variation in body size than their non-tropical counterparts (Read et al., 2018), and parental feeding and brooding behaviors varies across populations in orange-crowned warblers (Yoon et al., 2016). Future studies should span house sparrow populations at other latitudes, as well as other species who have historically experienced highly predictable developmental environments (temperature and parental behavior). These birds may be especially vulnerable to the effects of climate change.

There is still much work to be done in order to truly unravel the implications of what factors (temperature metrics and parental behavior across ontogeny) drive early and late avian developmental outcomes. This work presents not only a myriad of challenges, but also a multitude of exciting avenues for future study that could potentially inform conservation decisions.

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APPENDIX

Table A.1. Definitions of variables of interest in Chapter 2.

Variable	Type	Definition
Date	Explanatory variable for PCA	Julian day of incubation onset (the day of clutch completion)
Average ambient temperature	Explanatory variable for PCA	Average temperature of the entirety of incubation (from the last laid egg to the first hatched nestling) using hourly air temperatures obtained from NOAA
Variance in ambient temperature	Explanatory variable: fixed	Standard deviation around the mean squared for the hourly air temperatures (from NOAA) during the entirety of incubation (the time between the last laid egg and the first hatched nestling)
Minimum ambient temperature	Explanatory variable for PCA	The lowest recorded hourly air temperature (from NOAA) during incubation (the time between the last laid egg and the first hatched nestling)
Maximum ambient temperature	Explanatory variable for PCA	The highest recorded hourly air temperature (from NOAA) during incubation (the time between the last laid egg and the first hatched nestling)
Average nest temperature	Explanatory variable for PCA	Average temperature for the duration of the time the iButton was in the nest. The totality of the iButton readings (taken every 3 minutes while in the nest) were averaged.
Variance in nest temperature	Explanatory variable: fixed	Standard deviation around the mean squared for the duration of the time the iButton was in the nest. The totality of the iButton readings (taken every 3 minutes while in the nest) were included in the calculation.
Minimum nest temperature	Explanatory variable for PCA	The lowest recorded temperature for the duration of the time the iButton was in the nest. The totality of the iButton readings (taken every 3 minutes) were included.
Maximum nest temperature	Explanatory variable for PCA	The highest recorded temperature for the duration of the time the iButton was in the nest. The totality of the iButton readings (taken every 3 minutes) were included.
Seasonal thermal profile	Explanatory variable: fixed	The predicted values of the first PC from the PCA. Traits utilized included average ambient temperature, min ambient temperature, max ambient temperature, average nest temperature, min nest temperature, max nest temperature and date.
Clutch size	Explanatory variable: fixed	The maximum number of eggs in the nest
Brood size	Explanatory variable: fixed	The maximum number of nestlings in the nest
Treatment	Explanatory variable: fixed	After day 2 post-hatching, some nests were placed into treatment groups for a different study. Natural variation nests were not disturbed other than for previously described sampling. Stressed chicks were removed from the nest for an hour to simulate parental neglect. Control chicks were the siblings of the stressed chicks that were left in the nest during the stress treatment.
Assay	Explanatory variable: fixed	Which qPCR assay the telomere sample was run in.
Nest	Explanatory variable: random	The nest containing the iButton and nestlings.

Table A.1. Definitions of variables of interest in Chapter 2 (continued).

Variable	Type	Definition
Incubation period length	Response variable	The date of the last laid egg subtracted from the date of the first hatched nestling in a particular nest (days).
Day 2 body mass	Response variable	How much a nestling weighed at two days post-hatching (g).
Day 10 body mass	Response variable	How much nestling weighed at ten days post-hatching (g)
Day 2 telomere length	Response variable	Relative telomere length (T/S) from red blood cells collected from the nestling at day 2 post-hatching.
Day 10 telomere length	Response variable	Relative telomere length (T/S) from red blood cells collected from the nestling at day 10 post-hatching.
Hatching survival	Response variable	A binary measure of whether an individual survived to hatching. Birds that survived to hatching were assigned a 1 and birds that did not survive to hatching were assigned a 0.
Day 10 survival	Response variable	A binary measure of whether an individual survived to day 10 post-hatching. Birds that survived to hatching were assigned a 1 and birds that did not survive to hatching were assigned a 0.

Table A.2. Results of analyses used in model building.

Variable 1	Variable 2	r	t value	df	p value
Date	Average ambient temperature	**0.79**	9.96	58	< 0.0001 *
Date	Variance in ambient temperature	-0.25	-2.00	58	0.049 *
Date	Minimum ambient temperature	**0.83**	11.63	58	< 0.0001 *
Date	Maximum ambient temperature	**0.64**	6.36	58	< 0.0001 *
Average ambient temperature	Average nest temperature	**0.58**	5.46	58	< 0.0001 *
Variance in ambient temperature	Variance in nest temperature	0.098	0.75	58	0.45
Minimum ambient temperature	Minimum nest temperature	**0.77**	9.35	58	< 0.0001 *
Maximum ambient temperature	Maximum nest temperature	**0.52**	4.75	58	< 0.0001 *
Date	Average nest temperature	**0.54**	4.97	58	< 0.0001 *
Date	Variance in nest temperature	-0.10	-0.79	58	0.43
Date	Minimum nest temperature	**0.73**	8.19	58	< 0.0001 *
Date	Maximum nest temperature	**0.57**	5.29	58	< 0.0001 *

Table A.2. Results of analyses used in model building (continued).

Response Variable	Effects	Value	SE	DF	t value	p value
Day 10 mass	(Intercept)	23.54	0.89	114	26.19	< 0.0001 *
	Treatment: natural variation	-3.84	1.24	42	-3.07	**0.0037 ***
Model R^2 = 0.74	Treatment: stress	-0.66	0.53	114	-1.24	0.21
Day 10	(Intercept)	1.01	0.046	99	21.88	< 0.0001 *
telomere length	Treatment: natural variation	0.080	0.061	36	1.30	0.20
Model R^2 = 0.033	Treatment: stress	-0.018	0.063	99	-0.29	0.76
Response Variable	Effects	Estimate	SE	DF.r[+]	z value	p value
Day 10 survival	(Intercept)	2.07	0.76	211	2.69	0.0071 *
	Treatment: natural variation	-0.88	0.89	211	-0.98	0.32
Model R^2 = 0.54	Treatment: stress	0.075	0.63	211	0.11	0.90

[+] DF.r = residual degrees of freedom

Table A.3. Summary of trait loadings from the PCA, with cumulative variance explained by each PC given in parentheses.

	PC1 (74.9%)	PC2 (89.0%)	PC3 (94.5%)	PC4 (97.6%)	PC5 (99.1%)	PC6 (99.7%)	PC7 (100%)
Date	-0.84	-0.19	0.43	0.20	0.092	0.047	-0.0080
Average ambient temperature	-0.92	-0.33	-0.10	0.014	-0.11	-0.035	-0.11
Average nest temperature	-0.81	0.54	-0.076	-0.040	-0.083	0.13	-0.012
Minimum nest temperature	-0.89	0.18	0.15	-0.35	0.10	-0.053	-0.0078
Minimum ambient temperature	-0.92	-0.31	0.038	-0.045	-0.17	-0.017	0.10
Maximum nest temperature	-0.79	0.54	-0.090	0.22	0.016	-0.10	0.016
Maximum ambient temperature	-0.84	-0.32	-0.37	0.028	0.17	0.038	0.027

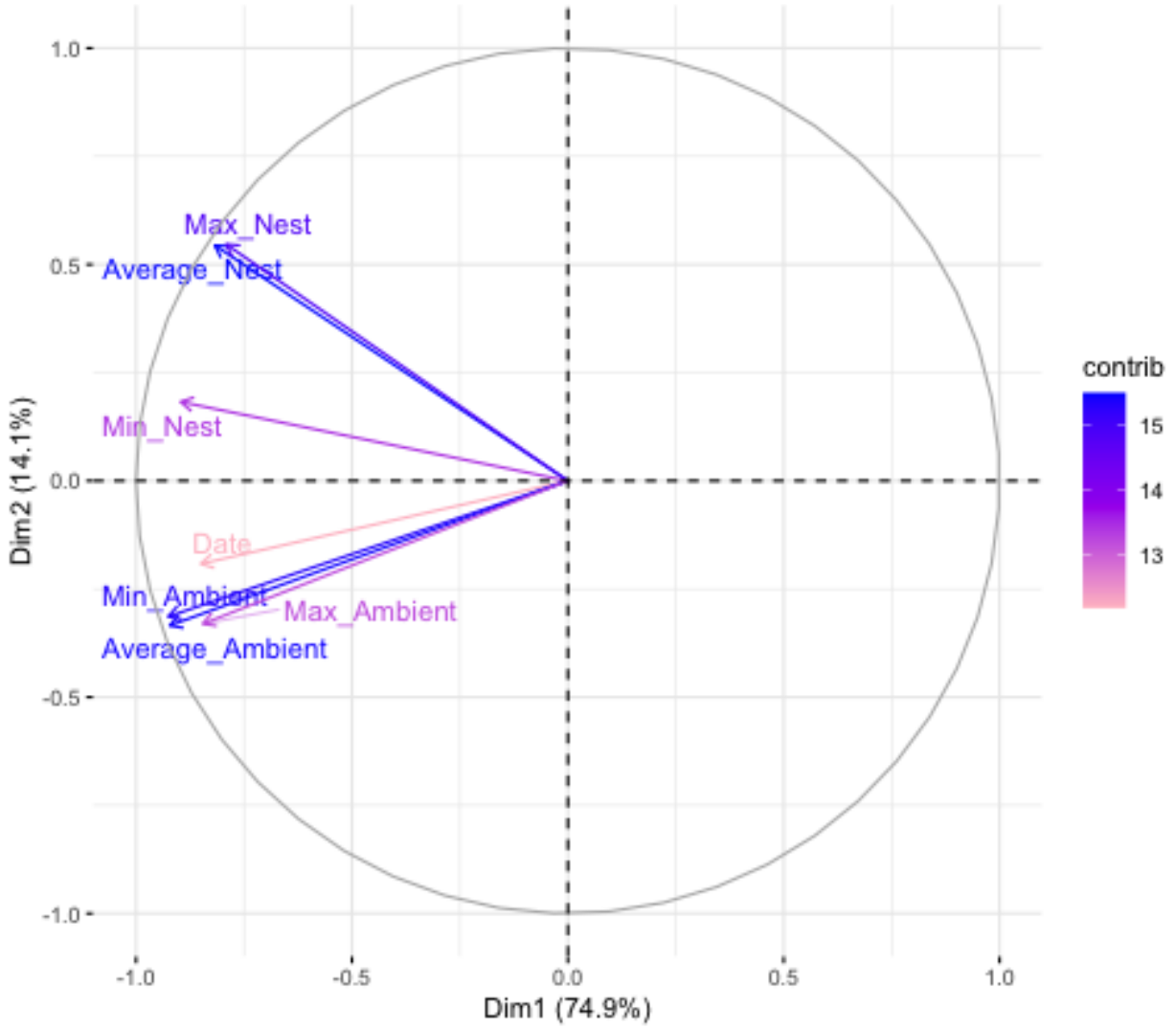

Figure A.1. Plot of trait contributions towards PC1 and PC2.

Table A.4. List of models utilized in the statistical analysis.

Type of Model	Response Variable	Fixed Effects	Random Effects
Linear model	Incubation period length	Pc1 + variance in ambient temperature + variance in nest temperature + clutch size	N/A
Binomial generalized linear mixed model	Hatching survival	Pc1 + variance in ambient temperature + variance in nest temperature + clutch size	Nest
Linear mixed effects model	Day 2 body mass	Pc1 + variance in ambient temperature + variance in nest temperature + clutch size	Nest
Linear mixed effects model	Day 2 telomere length	Pc1 + variance in ambient temperature + variance in nest temperature + clutch size + assay	Nest
Linear mixed effects model	Day 10 body mass	Pc1 + variance in ambient temperature + variance in nest temperature + brood size + treatment	Nest
Linear mixed effects model	Day 10 telomere length	Pc1 + variance in ambient temperature + variance in nest temperature + brood size + assay	Nest
Binomial generalized linear mixed model	Day 10 survival	Pc1 + variance in ambient temperature + variance in nest temperature + brood size	Nest

Table A.5. Ethogram of parental behaviors.

Behavior	Explanation
Enter	More than half the bird goes into the nest box
Remain	The entire bird is inside the box
Depart	The bird protrudes its head or additional body parts from the box but does not exit the box
Exit	The entire bird leaves the box

Table A.6. Defining variables of interest for Chapter 3.

Variable	Type	Definition
Date	Explanatory variable: fixed	Julian day of incubation onset (the day of clutch completion)
Average ambient temperature during incubation	Explanatory variable: fixed	Average temperature of the entirety of incubation (from the last laid egg to the first hatched nestling) for a nest using hourly air temperatures obtained from NOAA
Average ambient temperature during postnatal growth	Explanatory variable: fixed	Average temperature between the date the first nestling in the nest hatched and day 10 post-hatching using hourly air temperatures obtained from NOAA
Incubation attentiveness (male and female)	Explanatory variable: fixed	How many minutes per hour the parent spends fully inside the nest during an incubation video. The raw time was divided by the total run time of the video and then multiplied by 60.
Postnatal attentiveness (male and female)	Explanatory variable: fixed	How many minutes per hour the parent spends fully inside the nest during a postnatal video. The raw time was divided by the total run time of the video and then multiplied by 60.
Postnatal visits (male and female)	Explanatory variable: fixed	How many times per hour a parent enters a nest. The raw number of visits was divided by the total runtime of the video and then multiplied by 60.
Clutch size	Explanatory variable: fixed	The maximum number of eggs in the nest

Table A.6. Defining variables of interest for Chapter 3 (continued).

Variable	Type	Definition
Brood size	Explanatory variable: fixed	The maximum number of nestlings in the nest
Treatment	Explanatory variable: fixed	After day 2 post-hatching, some nests were placed into treatment groups for a different study. Natural variation nests were not disturbed other than for previously described sampling. Stressed chicks were removed from the nest for an hour to simulate parental neglect. Control chicks were the siblings of the stressed chicks that were left in the nest during the stress treatment.
Assay	Explanatory variable: fixed	Which qPCR assay the telomere sample was run in.
Nest	Explanatory variable: random	Which nest the iButton was placed in and that the nestlings were in.
Incubation period length	Response variable	The date of the last laid egg subtracted from the date of the first hatched nestling in a particular nest (days).
Day 2 body mass	Response variable	How much a nestling weighed at two days post-hatching (g).
Day 10 body mass	Response variable	How much nestling weighed at ten days post-hatching (g)
Day 2 telomere length	Response variable	Relative telomere length (T/S) from red blood cells collected from the nestling at day 2 post-hatching.
Day 10 telomere length	Response variable	Relative telomere length (T/S) from red blood cells collected from the nestling at day 10 post-hatching.
Hatching survival	Response variable	A binary measure of whether an individual survived to hatching. Birds that survived to hatching were assigned a 1 and birds that did not survive to hatching were assigned a 0.
Day 10 survival	Response variable	A binary measure of whether an individual survived to day 10 post-hatching. Birds that survived to hatching were assigned a 1 and birds that did not survive to hatching were assigned a 0.

Table A.7. Analyses utilized in model building

Response Variable	Explanatory Variables	Treatments	Value	SE	DF	t value	p value
Day 10 mass	Treatment +	(Intercept)	23.62	0.48	109	49.18	< 0.0001 *
	(1|nest)	Treatment: natural variation	2.76	0.72	109	3.83	0.0002 *
Model R^2 = 0.53		Treatment: stress	-0.60	0.46	109	-1.28	0.20
Day 10 telomere	Treatment +	(Intercept)	0.95	0.050	105	18.73	< 0.0001 *
length	(1|nest)	Treatment: natural variation	0.30	0.073	105	4.10	0.0001 *
Model R^2 = 0.24		Treatment: stress	0.097	0.062	105	1.56	0.12
Response Variable	Explanatory Variable	Treatments	Estimate	SE	DF.r+	z value	p value
Day 10 survival	Treatment +	(Intercept)	2.64	0.94	175	2.79	0.00519 *
	(1|nest)	Treatment: natural variation	0.16	1.033	175	0.16	0.87
Model R^2 = 0.48		Treatment: stress	0.057	0.61	175	0.093	0.92

Table A.7. Analyses utilized in model building (continued).

Variable 1	Variable 2	t value	df	r	p value
Date	Average ambient temperature during incubation	7.58	47	**0.74**	< 0.0001 *
Date	Average ambient temperature during postnatal growth	1.87	39	0.28	0.067
Average ambient temperature during incubation	Average ambient temperature during postnatal growth	5.51	39	**0.66**	< 0.0001 *
Average ambient temperature during incubation	Female incubation attentiveness	-2.35	47	-0.32	0.022 *
Date	Female incubation attentiveness	-2.19	54	-0.28	0.032 *
Average ambient temperature during incubation	Male incubation attentiveness	-0.51	47	-0.088	0.54
Date	Male incubation attentiveness	1.04	54	0.14	0.30
Average ambient temperature during postnatal growth	Female postnatal attentiveness	-1.27	39	-0.20	0.20
Date	Female postnatal attentiveness	1.87	39	0.29	0.068
Average ambient temperature during postnatal growth	Male postnatal attentiveness	3.18	39	0.45	0.0028 *
Date	Male postnatal attentiveness	1.34	39	0.21	0.18
Average ambient temperature during postnatal growth	Female postnatal visits	-2.08	39	-0.31	0.043 *
Date	Female postnatal visits	-1.27	39	-0.19	0.21
Average ambient temperature during postnatal growth	Male postnatal visits	1.32	39	0.20	0.19
Date	Male postnatal visits	-1.55	39	-0.23	0.14
Female incubation attentiveness	Male incubation attentiveness	-1.40	54	-0.18	0.16
Female postnatal attentiveness	Male postnatal attentiveness	-0.10	39	-0.016	0.91
Female postnatal visits	Male postnatal visits	-0.27	39	-0.044	0.78

[+] DF.r = residual degrees of freedom

Table A.8. Date and temperature models built and selected using AIC values.

Response Variable	Model Type	Explanatory Variables: T Model	Explanatory Variables: D Model	AIC: T Model	AIC: D Model	Model Selected
Female incubation attentiveness	Linear model	Average ambient temperature during incubation + clutch size	Date + clutch size	395.62	400.44	T
Male incubation attentiveness	Linear model	Average ambient temperature during incubation + clutch size	Date + clutch size	387.68	386.54	B
Female postnatal attentiveness	Linear model	Average ambient temperature during postnatal growth + brood size	Date + brood size	295.29	295.377	B
Male postnatal attentiveness	Linear model	Average ambient temperature during postnatal growth + brood size	Date + brood size	266.46	264.91	B

Table A.8. Date and temperature models built and selected using AIC values (continued).

Response Variable	Model Type	Explanatory Variables: T^{+} Model	Explanatory Variables: D^{++} Model	AIC: T Model	AIC: D Model	Model Selected
Female postnatal visits	Linear model	Average ambient temperature during postnatal growth + brood size	Date + brood size	255.29	253.77	B
Male postnatal visits	Linear model	Average ambient temperature during postnatal growth + brood size	Date + brood size	262.29	261.09	B
Incubation period length	Linear model	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + clutch size	Female incubation attentiveness + male incubation attentiveness + date + clutch size	134.99	139.24	T
Hatching survival	Binomial GLMM	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + clutch size + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + clutch size + (1|nest)	267.2	266.6	B
Day 2 body mass	Linear mixed-effects model	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + clutch size + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + clutch size + (1|nest)	518.88	524.34	T
Day 2 telomere length	Linear mixed-effects model	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + clutch size + assay + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + clutch size + assay + (1|nest)	154.06	157.44	T
Day 10 body mass	Linear mixed-effects model	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + treatment + brood size + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + treatment + brood size + (1|nest)	736.44	740.35	T
Day 10 body mass	Linear mixed-effects model	Female postnatal attentiveness + male postnatal attentiveness + average temperature during postnatal growth + treatment + brood Size + (1|nest)	Female postnatal attentiveness + male postnatal attentiveness + date + treatment + brood Size + (1|nest)	711.22	713.40	T
Day 10 body mass	Linear mixed-effects model	Female postnatal visits + male postnatal visits + average ambient temperature during postnatal growth + treatment + brood size + (1|nest)	Female postnatal visits + male postnatal visits + date + treatment + brood size + (1|nest)	709.19	712.99	T
Day 10 telomere length	Linear mixed-effects model	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + treatment + brood size + assay + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + treatment + brood size + assay + (1|nest)	145.20	147.47	T

Table A.8. Date and temperature models built and selected using AIC values (continued).

Response Variable	Model Type	Explanatory Variables: T Model	Explanatory Variables: D Model	AIC: T Model	AIC: D Model	Model Selected
Day 10 telomere length	Linear mixed-effects model	Female postnatal attentiveness + male postnatal attentiveness + average ambient temperature during postnatal growth + treatment + brood size + assay + (1|nest)	Female postnatal attentiveness + male postnatal attentiveness + date + treatment + brood size + assay + (1|nest)	142.73	146.38	T
Day 10 telomere length	Linear mixed-effects model	Female postnatal visits + male postnatal visits + average ambient temperature during postnatal growth + treatment + brood size + assay + (1|nest)	Female postnatal visits + male postnatal visits + date + treatment + brood size + assay + (1|nest)	143.89	149.44	T
Day 10 survival	Binomial GLMM	Female incubation attentiveness + male incubation attentiveness + average ambient temperature during incubation + brood size + (1|nest)	Female incubation attentiveness + male incubation attentiveness + date + brood size + (1|nest)	146.1	144.7	B
Day 10 survival	Binomial GLMM	Female postnatal attentiveness + male postnatal attentiveness + average ambient temperature during postnatal growth + brood size + (1|nest)	Female postnatal attentiveness + male postnatal attentiveness + date + brood size + (1|nest)	121.0	120.3	B
Day 10 survival	Binomial GLMM	Female postnatal visits + male postnatal visits + average ambient temperature during postnatal growth + brood size + (1|nest)	Female postnatal visits + male postnatal visits + date + brood size + (1|nest)	115.6	114.5	B

[+] T = Temperature
[++] D = Date