Sex-Specific Effects of Incubation Temperature on Embryonic Development of Zebra Finch (*Taeniopygia guttata*) Embryos

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ABSTRACT

In oviparous species, the embryonic environment—particularly temperature—can alter phenotype and survival of an individual by affecting its size as well as its metabolic rate. Previous studies have shown that incubation temperatures can affect sex ratio in birds; specifically, low incubation temperatures were shown to produce a male-biased sex ratio in zebra finches (*Taeniopygia guttata*) possibly because of a higher pre- or postnatal mortality rate in females. We hypothesized that sexes respond differently to suboptimal incubation temperature, leading to a male-biased sex ratio. To test this hypothesis, zebra finch eggs were incubated at 36.1°, 37.5°, or 38.5°C and hatching success, hatchling mass, residual yolk mass, and pectoralis mass were measured. We found that while hatchling mass was similar between the sexes at 37.5°C, female hatchlings were heavier at 36.1°C, and male hatchlings were heavier at 38.5°C. Pectoralis muscle mass was similar between the sexes at 36.1°C; however, at 37.5°C, female pectoralis mass was heavier at hatching than that of males. Females at 37.5°C also had lower residual yolk at hatching compared with males, reflecting a higher use of energy by female embryos compared with male embryos at this temperature. In contrast, residual yolk was similar between the sexes at 36.1° and 38.5°C. Our results suggest that there are sex differences in how incubation temperature alters organ mass and yolk energy reserve; this can lead to a difference in survival at different incubation temperatures between the sexes. Taken together with previous studies showing that females alter incubation behavior with ambient temperature, rising ambient temperatures could impact phenotype and survival of avian offspring in a sex-specific manner.

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Introduction

Fluctuations in the environment can have dramatic effects on developing young. Such phenotypic responses to the changing environment can impact potential survival and reproduction of the animal (Lindström 1999; Naguib and Gil 2005; Naguib et al. 2006; Gagliano and McCormick 2009). For instance, environmental temperature dictates sexual differentiation in species with temperature sex determination, such as turtles, crocodilians, and other reptiles (Pieau et al. 1999; Shine 1999; Valenzuela 2004). In reptiles with no direct control of incubation temperature, incubation temperature also affects offspring phenotype, including body size, growth, sprint speed, reproductive success, and survival (Parker and Andrews 2007; Warner and Shine 2008; DuRant et al. 2013; Dayananda et al. 2017).

In contrast to reptiles, in most birds egg temperature is highly regulated through incubation behavior; however, females have been shown to change their incubation behavior in response to changes in ambient temperature, resulting in changes to egg temperature (Conway and Martin 2000; Ardia et al. 2009, 2010). For instance, tree swallow (*Tachycineta bicolor*) females of experimentally cooled nests spent less time incubating eggs, leading to egg temperatures that were lower than control nests (Ardia et al. 2010). Nord et al. (2010) manipulated clutch size in addition to ambient temperature in zebra finches (*Taeniopygia guttata*), finding significantly higher clutch temperatures (measured just below the eggs) at 28°C compared with 20° or 10°C. Clutch temperatures also lowered significantly when the female was incubating six eggs instead of four. In response to a change in ambient temperature, incubation behavior, or clutch size, egg temperature may deviate from what is optimal for embryonic development. This can have short- and long-term consequences on offspring phenotype, survival, and sex ratio in species with genotypic sex determination, such as wood ducks (*Aix sponsa*; DuRant et al. 2010, 2012*a*, 2012*b*, 2016; Hopkins et al. 2011), megapodes (Goth and Booth 2005; Eiby et al. 2008; Eiby and Booth 2009), and zebra finches (Wada et al. 2015, forthcoming). In megapodes, which use heat from decomposing plant matter to incubate eggs, higher incubation temperatures cause a female-biased sex ratio because of high male embryonic mortality, while lower incubation temperatures result in a male-biased sex ratio because of high female mortality (Goth and Booth 2005; Eiby et al. 2008). Studies on precocial birds also show that young hatched from low incubation temperatures have

higher adrenocortical responses, slower locomotor performance, and higher metabolic rates during a thermal challenge compared with young hatched from middle or high incubation temperatures (DuRant et al. 2010, 2012*b*; Hopkins et al. 2011). Similar to precocial birds, suboptimal clutch temperatures have been shown to significantly affect the long-term survival of the altricial zebra finch; eggs incubated at the suboptimal temperature of 39.5°C had significantly lower long-term survival compared with eggs incubated at 37.9°C (Berntsen and Bech 2016). Recently, it has been shown that a low incubation temperature of 36.2°C produced a male-biased sex ratio in zebra finches (Wada et al., forthcoming). However, the sex ratio was determined via plumage examination in the study; thus, when and how the skewed sex ratio arose remains unknown.

One possible mechanism underlying sex-specific mortality rates is variation in energy requirement for growth and survival. Avian embryos are ectothermic, although the pattern of thermoregulatory ability differs between precocial and altricial species (Wada et al. 2018). Numerous studies indicate that embryos incubated at lower temperatures take longer to hatch and expend more energy than those at higher temperatures (Booth 1987; DuRant et al. 2012*a*, 2012*b*; Wada et al. 2015). DuRant et al. (2011) showed that while energy expenditure before external pipping was similar across all incubation temperature groups, wood duck embryos incubated at low temperature expended the most energy during pipping compared with embryos incubated at middle or high temperatures. As a result, embryos incubated at low temperatures had the highest total energy expenditure during the incubation period, with the longest incubation duration compared with embryos incubated at middle or high temperatures (DuRant et al. 2011; see also Hepp et al. 2006). Consequently, hatchlings from low incubation temperatures are estimated to have the lowest remaining energy. Similarly, zebra finch embryos that experienced period cooling had lower residual yolk on day 12 of development compared with eggs incubated at a constant optimal temperature (Olson et al. 2006). Since hatchlings rely on yolk reserve for the first couple of days, variation in residual yolk at hatching likely impacts posthatch growth and survival of oviparous embryos (DuRant et al. 2011). Incubation temperature also influences nestling and fledgling metabolic rates in altricial avian species. For example, blue tit (*Cyanistes caeruleus*) nestlings from eggs incubated at lower temperatures had a significantly higher resting metabolic rate than those incubated at higher temperatures (Nord and Nilsson 2011). Similarly, zebra finch fledglings from eggs incubated at low temperatures had significantly higher resting metabolic rates than those from eggs incubated at high or control temperatures (Wada et al. 2015). Interestingly, this effect of incubation temperature on postnatal metabolic rates in zebra finches was seen only in females. These studies show that a lower than optimal incubation temperature increases energy expenditure during pre- and postnatal periods. Because yolk provides a finite amount of resources for embryonic and early postnatal development in birds, if sexes differ in energy expenditure in response to suboptimal incubation temperature during or after the embryonic period, this may lead to sex-specific mortality at those temperatures.

On the basis of previous studies that showed low incubation temperature elevated metabolic rates and skewed sex ratios of zebra finches toward males, we hypothesized that there are sex differences in mass of metabolically demanding organs and yolk consumption. In order to test this hypothesis, we incubated zebra finch embryos at three different temperatures (36.1°, 37.5°, and 38.5°C) and measured hatching success, body mass, residual yolk, and pectoralis mass of hatchlings. These incubation temperatures are within the range of naturally occurring incubation temperature (Zann and Rossetto 1991) and similar to those used by Wada et al (2015), where 37.4°C yielded the highest overall survival. We predicted that (1) low and high incubation temperatures skew sex ratio toward males during the embryonic development and (2) females incubated at low and high incubation temperature weigh more and have larger pectoralis and higher yolk consumption than males, leading to lower residual yolk at hatching.

Methods

Animal Husbandry

Twenty-four adult zebra finch pairs from the breeding colony at the Avian Research Laboratory at Auburn University were placed in individual cages (38.1 cm × 45.72 cm × 45.72 cm) and provided with nest boxes (19.05 cm × 13.55 cm × 13.55 cm) and material to construct nests. Because zebra finches in the arid regions of Australia use rainfall as an environmental cue to initiate breeding (Frith and Tilt 1959; Davies 1977; Zann et al. 1995; Zann 1999), each pair was sprayed daily with water to stimulate breeding. The room housing each breeding pair was maintained on a 14L:10D cycle at 22°C. All pairs were provided access to seed and cuttlefish bone ad lib. In addition, pairs were provided with a mixture of hardboiled chicken eggs, cornmeal, and white bread daily. Before pairing, the male and female of each pair were weighed (nearest 0.01 g).

Egg Collection and Incubation

Egg checks of each pair were performed daily. Any new eggs were removed, labeled, and weighed to obtain an initial egg mass. The eggs were then allocated (on the same day of laying) to one of three incubators in a systematic fashion to allow eggs from each pair to be parsed among the three temperature treatments while balancing laying order among treatment groups.

A total of 215 eggs were used in this study. Eggs were incubated in Brinsea Octagon 20 Advance EX incubators (Brinsea, Titusville, FL) until hatching at one of three incubation temperatures: 36.1° ($n = 65$), 37.5° ($n = 72$), or 38.5°C ($n = 78$). Each incubator maintained a relative humidity of 55%. Beginning on day 9 of incubation, eggs were checked three times (0700, 1400, and 2000 hours) daily for hatchlings. On discovery of a hatchling, the time of discovery was noted, and the individual was removed from the incubator and placed at 37°C until euthanasia.

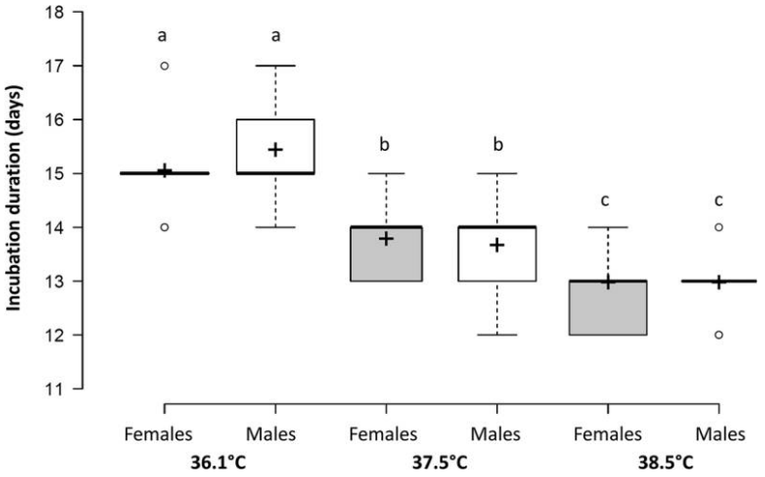

Figure 1. Incubation duration for zebra finch eggs incubated at 36.1°, 37.5°, and 38.5°C. Incubation duration decreased as incubation temperature increased ($P < 0.001$). Eggs, on average, hatched after 15.2 d (36.1°C), 13.6 d (37.5°C), and 12.9 d (38.5°C). Thick lines show the medians, crosses show the sample means, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and circles represent outliers. Different letters indicate significant differences between groups ($P \geq 0.05$); $n = 17, 13, 11, 13, 13, 13$.

Euthanasia, Dissection, and Tissue Weights

Immediately before euthanasia, all hatchlings were weighed (nearest 0.001 g). Hatchlings were then euthanized with a lethal dose of isoflurane and cervical dislocation. Within 10 min of euthanasia, both the left and right pectoralis muscles were collected and weighed (nearest 0.001 g), and the remaining yolk was separated from the hatchling and weighed (nearest 0.001 g). Remaining tissues were placed at −20°C until DNA extraction for sexing.

Unhatched eggs were candled to determine fertility. Infertile eggs were noted and discarded. Embryos that died in ovo were dissected to determine stage of development at death (early, early–mid, mid, mid–late, late development or pipping), and tissues were placed at −20°C until sexing.

Sexing

To determine hatchling sex, DNA from the right or left wing of each individual was extracted using a DNAeasy kit (Qiagen, Hilden) following the manufacturer's specific protocol for tissue samples. Arms were also used to extract DNA from mid- and late-development embryos, whereas the entire embryo was used for DNA extraction of earlier staged individuals. DNA was not able to be extracted from all embryos that died very early in development. After extraction, samples were stored at −20°C until concentration was determined (NanoDrop 2000, ThermoFisher Scientific, Waltham, MA). Samples were then diluted to a final concentration of 20 ng/μL.

A polymerase chain reaction (PCR) was performed using the primers specified by Soderstrom et al. (2007). Briefly, a PCR was performed in a total volume of 25 μL consisting of four primers at a final concentration 1 μM each (W1, W2, Z2, and Z2), 1 × PCR Master Mix (M7505, Promega, Madison, WI), and 2.5 μL of genomic DNA from each sample. The PCR was performed on a PCT 100 thermal cycler (Bio-Rad Laboratories, Hercules, CA) following the conditions of Soderstrom et al. (2007).

Following PCR, products were subsequently run on a 1.2% agarose gel electrophoresis with SYBR Safe (1:25,000 dilution in 1 × TBE) for 45 min in the dark and visualized on an ImageQuant LAS4000 (GE Healthcare, Pittsburgh). Sex of each sample was called on the basis of the banding patterns of individuals of known sex (i.e., adults; see Soderstrom et al. 2007).

Statistical Analysis

All statistical analyses were performed using SPSS 21. The effects of incubation temperature (36.1°, 37.5°, or 38.5°C) and sex on incubation duration, hatchling mass, pectoralis mass, and yolk mass remaining at hatching were analyzed using a linear mixed model. Only individuals with sex data were included in the statistical analysis. Final sample sizes for males and females were 12 and 15 for 36.1°C, 9 and 9 for 37.5°C, and 13 and 13 for 38.5°C.

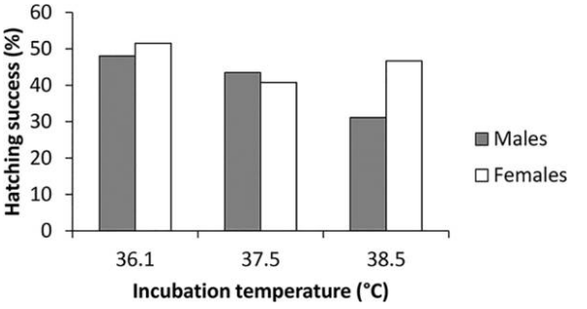

Figure 2. Percent of successful hatching of females and males from each treatment group. Hatchling success was not affected by sex ($P = 0.63$) or incubation temperature ($P = 0.34$).

Since multiple individuals in this study were siblings, nest number (signifying the two parents of the offspring) was used as a random effect. Initial egg mass was used as a covariate when significant to account for eggs of differing sizes at laying for analyzing hatchling mass. Because we aimed to test whether incubation temperature or sex influenced a proportion of yolk and pectoralis mass relative to the hatchling mass, we used hatchling mass as a covariate for yolk and pectoralis mass. Since not all hatching events were visually observed, time between when an egg was last observed (at one of the three observation times) until the hatchling was euthanized was used as a covariate when significant. Hatching success was analyzed using a logistic regression with nest as a random effect and sex and incubation temperature as fixed factors. Natural log transformation was used when outcome variables violated assumptions of normality; yolk mass was the only variable that was log transformed. Likewise, values were excluded if their residuals exceeded ± 4 SDs from the mean. Least square means adjusted for covariates in the statistical model $\pm$ standard errors are presented in the text. Natural log-transformed least squares means were back-transformed by e raising to a power. Raw values are shown in figures. Box plots were drawn using BoxPlotR, where thick lines show the medians, box limits indicate the 25th and 75th percentiles as determined by R software, whiskers extend 1.5 times the interquartile range from

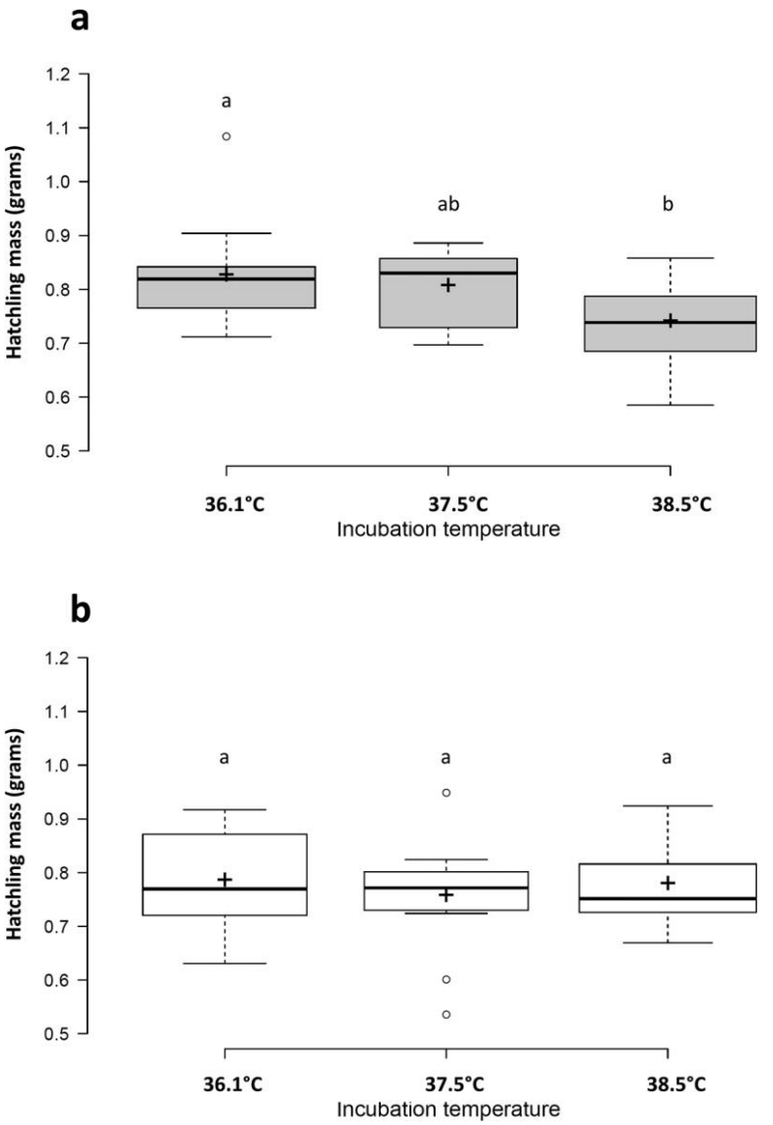

Figure 3. Hatchling mass of females (*a*) and males (*b*) from three incubation temperatures. Incubation temperature significantly affected hatchling mass only in female hatchlings (sex × incubation temperature: $P = 0.018$). Female hatchlings from 36.1°C were 0.082 g heavier than females from 38.5°C ($P = 0.001$). Thick lines show the medians, crosses show the sample means, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and circles represent outliers. Different letters indicate significant differences between groups ($P \geq 0.05$); $n = 15, 9, 14$ (females); $n = 12, 12, 14$ (males).

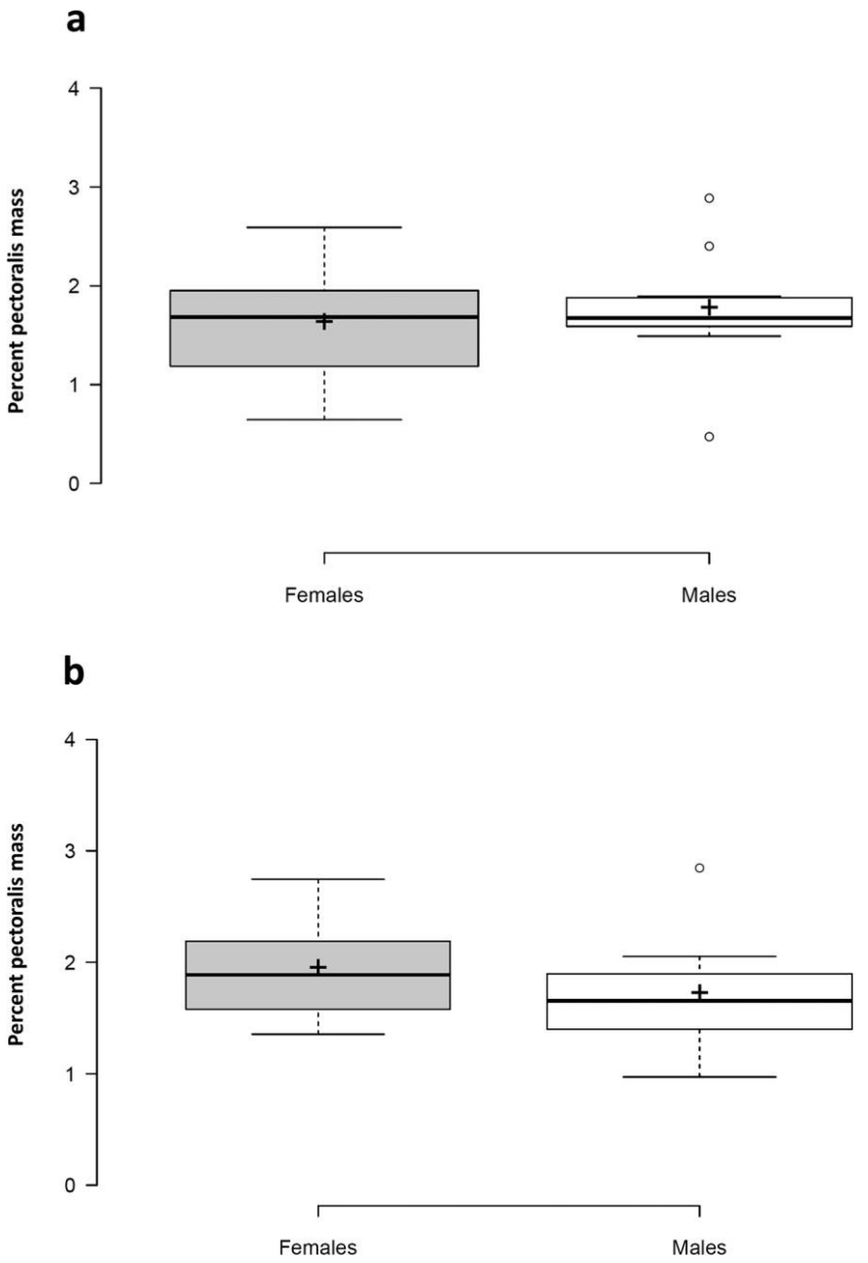

Figure 4. Percent pectoralis mass relative to hatchling mass of young from eggs incubated at 36.1° (*a*), 37.5° (*b*), and 38.5°C (*c*). Females had heavier pectoralis than males within the 37.5°C group ($P = 0.025$) and within the 38.5°C group ($P = 0.077$). Thick lines show the medians, crosses show the sample means, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and circles represent outliers. Different letters indicate significant differences between groups ($P \geq 0.05$); $n = 14$, 12 (36.1°C); $n = 9$, 12 (37.5°C); $n = 14$, 13 (38.5°C).

the 25th and 75th percentiles, circles represent outliers, and crosses indicate means. Significance was set at $\alpha = 0.05$.

Results

Incubation Duration

As expected, incubation duration was strongly affected by incubation temperature ($F = 71.38$, $P < 0.001$; fig. 1). Incubation duration, on average, was 15.2 ± 0.14 d in the 36.1°C group, 13.6 ± 0.16 d for the 37.5°C group, and 12.9 ± 0.14 d for the 38.5°C group. In other words, eggs in the 36.1°C group hatched 1.6 d later than those in the 37.5°C group, which hatched 0.7 d later than the 38.5°C group. There was no effect of sex ($F = 0.132$, $P = 0.71$) on incubation duration nor any interaction between incubation temperature and sex ($F = 1.30$, $P = 0.28$).

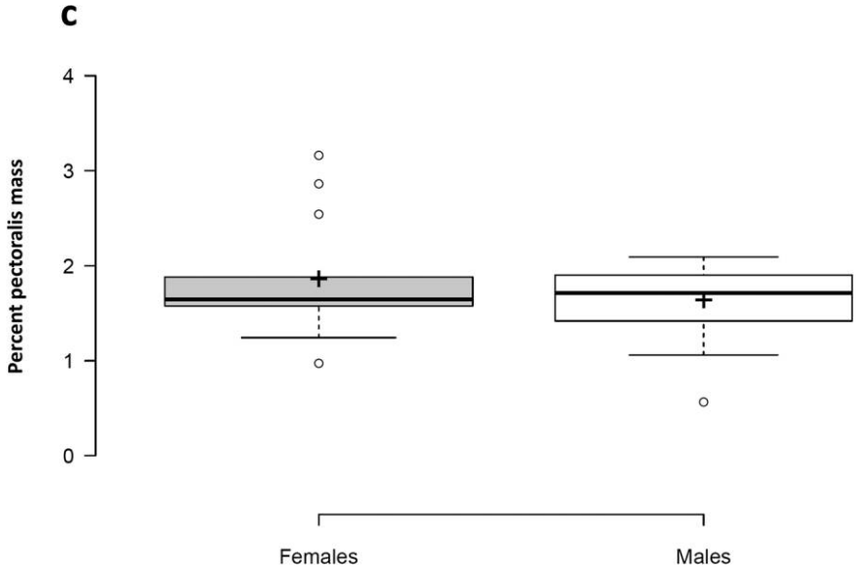

Figure 4 (*Continued*)

Hatching Success

Hatching success was not affected by sex or incubation temperature (sex: $F = 0.23$, $P = 0.63$; incubation temperature: $F = 1.09$, $P = 0.34$; fig. 2). Hatching success of embryos incubated at 36.1°, 37.5°, and 38.5°C was 46%, 42%, and 36%, respectively. Similarly, there was no significant interaction of sex and incubation temperature (sex × incubation temperature: $F = 0.13$, $P = 0.88$).

Hatchling Mass

Egg mass on day 0 of incubation was used as a covariate in the final model (egg mass on day 0: $F = 81.27$, $P < 0.001$). On average, male hatchlings weighed 0.774 ± 0.017, 0.750 ± 0.020, and 0.779 ± 0.015 g, while female hatchlings weighed 0.818 ± 0.015, 0.776 ± 0.019, and 0.736 ± 0.015 g in the 36.1°, 37.5°, and 38.5°C groups, respectively. Hatchling mass of males and females differed depending on the incubation temperature (sex: $F = 0.45$, $P = 0.50$; incubation temperature: $F = 3.36$, $P = 0.04$; sex × incubation temperature: $F = 4.25$, $P = 0.018$; fig. 3). When eggs were incubated at 37.5°C, hatchling mass did not differ between sexes. When eggs were incubated at 38.5°C, males were 0.043 g heavier than females, although the difference was only statistically suggestive ($P = 0.055$). Similarly, when eggs were incubated at 36.1°C, females were 0.045 g heavier than males ($P = 0.051$). When hatch mass was compared within sex, female hatchlings from the 36.1°C incubator were 0.082 g heavier than female hatchlings from the 38.5°C incubator ($P = 0.001$). In contrast, there was no difference in hatch mass among incubation temperature groups for male hatchlings.

Pectoralis Mass

Hatchling mass and time lapse between hatching and euthanasia were used as covariates in the final model (hatchling mass: $F = 0.46$, $P = 0.50$; time: $F = 0.02$, $P = 0.89$; incubation temperature × time: $F = 3.77$, $P = 0.029$; sex × incubation temperature × hatchling mass: $F = 4.25$, $P = 0.003$). On average, pectoralis of male hatchlings weighed 13.62 ± 1.03, 11.73 ± 1.42, and 11.81 ± 1.05 mg, while that of female hatchlings weighed 14.77 ± 1.16, 16.59 ± 1.52, and 14.54 ± 1.08 mg in the 36.1°, 37.5°, and 38.5°C groups, respectively. Neither sex nor incubation temperature alone influenced pectoralis mass (sex: $F = 1.79$, $P = 0.19$; incubation temperature: $F = 0.84$, $P = 0.44$; fig. 4). However, there was a significant sex × incubation temperature interaction ($F = 7.88$, $P = 0.001$). Within each sex, there was no difference in pectoralis mass among the incubation temperature group ($P = 0.5$). In the 36.1°C group, female and male hatchlings had a similar pectoralis mass ($P = 0.46$). However, in the 37.5°C group, the pectoralis of female hatchlings was 4.85 mg heavier compared with male hatchlings ($P = 0.025$). Similarly, in the 38.5°C group, the pectoralis of female hatchlings was 2.73 mg heavier compared with male hatchlings, although this difference was not statistically significant ($P = 0.077$).

Remaining Yolk Mass at Hatching

Hatchling mass was used as a covariate in the final model of yolk mass ($F = 29.07$, $P < 0.001$). On average, residual yolk of male hatchlings weighed 63.82, 72.60, and 59.74 mg, while that of female hatchlings weighed 67.15, 57.28, and 70.39 mg in the 36.1°, 37.5°, and 38.5°C groups, respectively. Yolk mass at hatching was not affected by sex or incubation temperature alone (sex: $F = 0.014$, $P = 0.91$; incubation temperature: $F = 0.02$, $P = 0.98$; fig. 5). However, there was a significant interaction between sex and incubation temperature ($F = 3.43$, $P = 0.039$). Within each sex, there was no effect of incubation temperature on residual yolk mass at hatching ($P > 0.05$). When eggs were incubated at 37.5°C, the yolk mass of male embryos was 15.32 mg

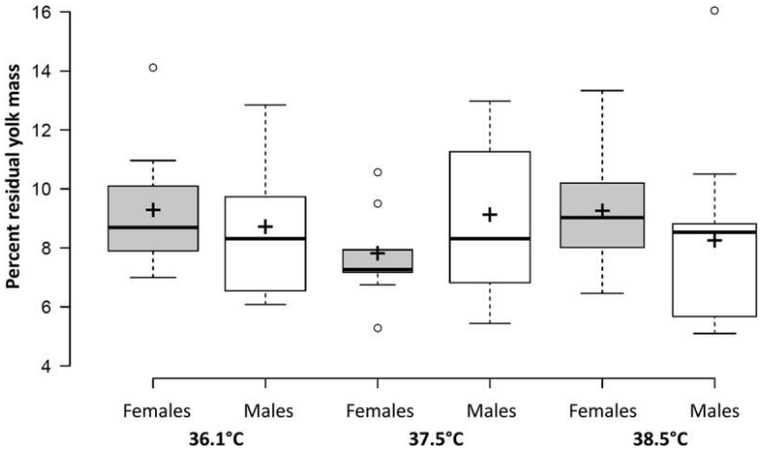

Figure 5. Percent yolk mass relative to hatchling mass. Yolk mass was not affected by sex or incubation temperature alone but was significantly affected by the sex × incubation temperature interaction ($P = 0.039$). At 37.5°C, yolk mass of male embryos was 15.32 mg heavier than that of females ($P = 0.051$); at 38.5°C, male residual yolk mass was marginally lighter than females ($P = 0.095$). Thick lines show the medians, crosses show the sample means, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and circles represent outliers; $n = 15, 12, 9, 12, 13, 13$.

heavier than the yolk mass of female embryos ($P = 0.051$). In contrast, when eggs were incubated at 38.5°C, the yolk mass of male embryos was 10.65 mg lighter than the yolk mass of female embryos, although the difference was not significant ($P = 0.095$).

Discussion

On the basis of our previous study, where incubation temperatures 1°C lower than what is considered as optimal resulted in a male-biased sex ratio in juvenile zebra finches (Wada et al., forthcoming), we hypothesized that incubation temperature influences growth rate in a sex-specific manner. Specifically, we hypothesized that females have higher energetic demands for growth and that this depletes yolk resources, leading to lower embryonic survival at suboptimal incubation temperatures compared with males. Thus, we predicted that females have heavier pectoralis muscles and higher body mass as well as lower residual yolk at hatching compared with males at suboptimal temperatures. The results from this study show that female hatchling mass declined with increasing incubation temperature, while male hatchling mass was not affected by incubation temperature. While we found no overall effect of temperature on pectoralis mass within each sex, female hatchlings incubated at 37.5°C had larger pectoralis muscles than males. Residual yolk mass at hatching also differed between sexes: at 37.5°C, yolk mass of female hatchlings was lower than yolk mass of male hatchlings.

There are three possibilities for why residual yolk at hatching differed between sexes. First, a greater amount of water loss may have led to lower residual yolk at hatching in one particular sex. It has been shown that eggs incubated at higher temperatures lose a higher percentage of egg mass during the first 12 d of incubation compared with eggs incubated at lower temperatures (Wada et al. 2015). However, the sex difference in residual yolk mass is within an incubation temperature treatment; thus, differential water loss is unlikely to explain this sex-specific effect. Second, yolk reserves may be converted to tissue growth and metabolism at a higher rate in one sex compared with the other. There were suggestive sex differences in hatchling weight and a significant effect of incubation temperature on female hatchling mass; however, male and female hatchlings incubated at 37.5°C weighed similarly. Thus, this does not explain the sex differences in residual yolk mass at 37.5°C. Last, it is possible that there is a sex difference in the amount of solid left behind at hatching or metabolic rate during the embryonic development. Because we did not quantify the solid materials left at hatching, further study is needed to test this.

Related to the amount of solid left behind at hatching, male and female embryos may differ in metabolic rate during the incubation period. Pectoralis muscle is among the largest and most energy-demanding muscles for birds (Marsh and Dawson 1989). Accordingly, pectoralis mass is positively correlated to the summit metabolic rate in some songbirds (Swanson et al. 2014). The larger pectoralis mass of females incubated at 37.5°C suggests that female embryos incubated at this temperature had a higher metabolic rate and higher energy consumption than males. In fact, embryos incubated at 37.5°C have the largest sex difference in residual yolk as well, with females having less residual yolk than males. This increased yolk consumption or low residual yolk at hatching may become an issue for oviparous embryos and hatchlings. Zebra finch parents do not usually begin feeding their young on the day of hatch (Zann 1996). This could have adverse effects on hatchlings with smaller residual yolk mass, resulting in a period of malnutrition after hatching that can affect development and may lead to early death.

Similar to previous findings, incubation duration was strongly affected by incubation temperature (DuRant et al. 2012*a*, 2012*b*;

Wada et al. 2015). However, we found no effect of incubation temperature on sex ratio. There are three possibilities behind the disparity in the effects of incubation temperature on sex ratio between this study and that of Wada et al. (forthcoming). First, the hatching success of this study was generally lower compared with that of Wada et al. (2015). The lower hatching success in this study may have hindered our ability to detect differences among temperature groups. The second possibility is that there may be population differences in sensitivity of embryos to the incubation temperature. In Asian yellow pond turtles (*Mauremys mutica*), hatching success and hatchling mass were affected by incubation temperature differently in low- and high-latitude populations (Zhao et al. 2015). There are no studies showing similar findings in zebra finches, but this raises the question about the possible population differences in sensitivity to embryonic environment among wild and captive zebra finches and other altricial passerines. This study used the same treatment protocol and the same model of incubators used by Wada et al. (2015, forthcoming). However, the colony used in this study was different from that of Wada et al. (2015, forthcoming). Thus, it is possible that the colony of birds used in this study was more responsive to transportation between the nest and an incubator and temperature manipulation in general. The third possibility is that the sex-specific mortality described by Wada et al. (2015, forthcoming) may have occurred after hatching in these previous studies. Wada et al. (2015) reported that incubation at 38.4°C resulted in the highest prehatch mortality (40.9% compared with 19.7% and 15.4% in the 36.2° and 37.4°C groups, respectively), while incubation at 36.2°C resulted in the highest posthatch mortality (17.5% compared with 5.2% and 0% in the 37.4° and 38.4°C groups, respectively). Since birds were visually sexed around 40 d after hatch by Wada et al. (forthcoming), it is possible that the male-biased sex ratio that occurred at 36.2°C was due to more female nestlings dying after hatching rather than sex-specific mortality during the embryonic period.

Although there was no effect of incubation temperature on sex ratio in this study, our results show sex-specific effects of incubation temperature on hatchling mass, suggesting that females may be more susceptible to the adverse effects of suboptimal incubation temperatures than males. Previous studies in birds have shown that during the embryonic period, males are more susceptible to developmental stressors than females (Hayward et al. 2006; Love and Williams 2008*a*, 2008*b*), while during the posthatch period, females are more susceptible to stressors (e.g., exogenous corticosterone, brood size manipulation, and nutritional stress) than males (Haywood and Perrins 1992; de Kogel 1997; Bradbury and Blakey 1998; Kilner 1998; Gorman and Nager 2004; Martins 2004; Verhulst et al. 2006; Schmidt et al. 2012; but see also Love et al. 2005; Love and Williams 2008*a*). For instance, in ovo injection of corticosterone caused male European starling nestlings to have lighter body weights compared with vehicle-injected males (Love and Williams 2008*a*, 2008*b*). However, female nestlings in the corticosterone and vehicle-injected groups weighed the same. Similarly, in ovo corticosterone injection slowed down growth of Japanese quail chicks in the first 8 d of hatch, but this was observed only in males (Hayward et al. 2006).

Contrary to previous studies, our results suggest that the prenatal thermal environment has a stronger effect on female embryos than male embryos. The results from this study show that hatchling mass was affected by incubation temperature only in female offspring. Females incubated at 37.5°C tended to have lower yolk mass at hatching compared with males. Furthermore, females incubated at higher temperatures had greater pectoralis mass than males in the same temperature. Because nest temperature is shown to covary with ambient temperature, even in birds that actively incubate their eggs (Ardia et al. 2010; Nord et al. 2010), large fluctuations in ambient temperatures could cause incubation temperatures to become suboptimal. If sexes differ in sensitivity to embryonic environment, this may influence hatchling phenotype, postnatal growth rate, and survival. Future research is necessary to test this hypothesis.

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Literature Cited

Ardia D.R., J.H. Perez, E.K. Chad, M.A. Voss, and E.D. Clotfelter. 2009. Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. J Anim Ecol 78:4–13.

Ardia D.R., J.H. Pérez, and E.D. Clotfelter. 2010. Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. Proc R Soc B 277:1881–1888.

Berntsen H.H. and C. Bech. 2016. Incubation temperature influences survival in a small passerine bird. J Avian Biol 47:141–145.

Booth D.T. 1987. Effect of temperature on development of mallee fowl *Leipoa ocellata* eggs. Physiol Zool 60:437–445.

Bradbury R.B. and J.K. Blakey. 1998. Diet, maternal condition, and offspring sex ratio in the zebra finch, *Poephila guttata*. Proc R Soc B 265:895–899.

Conway C.J. and T.E. Martin. 2000. Effects of ambient temperature on avian incubation behavior. Behav Ecol 11:178–188.

Davies S.J. 1977. The timing of breeding by the zebra finch *taeniopygia castanotis* at Mileura, Western Australia. Ibis 119: 369–372.

Dayananda B., S. Penfold, and J.K. Webb. 2017. The effects of incubation temperature on locomotor performance, growth and survival in hatchling velvet geckos. J Zool 303:46–53.

de Kogel C.H. 1997. Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. J Anim Ecol 66:167–178.

DuRant S.E., G.R. Hepp, I.T. Moore, B.C. Hopkins, and W.A. Hopkins. 2010. Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. J Exp Biol 213:45–51.

DuRant S.E., W.A. Hopkins, A.W. Carter, L.T. Kirkpatrick, K.J. Navara, and D.M. Hawley. 2016. Incubation temperature causes skewed sex ratios in a precocial bird. J Exp Biol 219: 1961–1964.

DuRant S.E., W.A. Hopkins, D.M. Hawley, and G.R. Hepp. 2012*a*. Incubation temperature affects multiple measures of immunocompetence in young wood ducks (*Aix sponsa*). Biol Lett 8:108–111.

DuRant S.E., W.A. Hopkins, and G.R. Hepp. 2011. Embryonic developmental patterns and energy expenditure are affected by incubation temperature in wood ducks (*Aix sponsa*). Physiol Biochem Zool 84:451–457.

DuRant S.E., W.A. Hopkins, G.R. Hepp, and J.R. Walters. 2013. Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. Biol Rev 88:499–509.

DuRant S.E., W.A. Hopkins, A.F. Wilson, and G.R. Hepp. 2012*b*. Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird. Funct Ecol 26: 416–422.

Eiby Y.A. and D. Booth. 2009. The effects of incubation temperature on the morphology and composition of Australian brush-turkey (*Alectura lathami*) chicks. J Comp Physiol B 179:875–882.

Eiby Y.A., J.W. Wilmer, and D.T. Booth. 2008. Temperature-dependent sex-biased embryo mortality in a bird. Proc R Soc B 275:2703–2706.

Frith H.J. and R.A. Tilt. 1959. Breeding of the zebra finch in the Murrumbidgee irrigation area, New South Wales. Emu Austral Ornithol 59:289–295.

Gagliano M. and M.I. McCormick. 2009. Hormonally mediated maternal effects shape offspring survival potential in stressful environments. Oecologia 160:657–665.

Gorman H.E. and R.G. Nager. 2004. Prenatal developmental conditions have long-term effects on offspring fecundity. Proc R Soc B 271:1923–1928.

Goth A. and D.T. Booth. 2005. Temperature-dependent sex ratio in a bird. Biol Lett 1:31–33.

Hayward L.S., J.B. Richardson, M.N. Grogan, and J.C. Wingfield. 2006. Sex differences in the organizational effects of corticosterone in the egg yolk of quail. Gen Comp Endocrinol 146: 144–148.

Haywood S. and C.M. Perrins. 1992. Is clutch size affected by environmental conditions during growth? Proc R Soc B 249: 195–197.

Hepp G.R., R.A. Kennamer, and M.H. Johnson. 2006. Maternal effects in wood ducks: incubation temperature influences incubation period and neonate phenotype. Funct Ecol 20:308–314.

Hopkins B.C., S.E. DuRant, G.R. Hepp, and W.A. Hopkins. 2011. Incubation temperature influences locomotor performance in young wood ducks (*Aix sponsa*). J Exp Zool 315A: 274–279.

Kilner R. 1998. Primary and secondary sex ratio manipulation by zebra finches. Anim Behav 56:155–164.

Lindström J. 1999. Early development and fitness in birds and mammals. Trends Ecol Evol 14:343–348.

Love O.P., E.H. Chin, K.E. Wynne-Edwards, and T.D. Williams. 2005. Stress hormones: a link between maternal condition and sex-biased reproductive investment. Am Nat 166:751–766.

Love O.P. and T.D. Williams. 2008*a*. The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. Am Nat 172:E135–E149.

———. 2008*b*. Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. Horm Behav 54:496–505.

Marsh R.L. and W.R. Dawson. 1989. Avian adjustments to cold. Pp. 206–253 in L.C.H. Wang, ed. Advances in comparative and environmental physiology. Vol. 4. Animal adaptation to cold. Springer, Berlin.

Martins T.L.F. 2004. Sex-specific growth rates in zebra finch nestlings: a possible mechanism for sex ratio adjustment. Behav Ecol 15:174–180.

Naguib M. and D. Gil. 2005. Transgenerational body size effects caused by early developmental stress in zebra finches. Biol Lett 1:95–97.

Naguib M., A. Nemitz, and D. Gil. 2006. Maternal developmental stress reduces reproductive success of female offspring in zebra finches. Proc R Soc B 273:1901–1905.

Nord A. and J.A. Nilsson. 2011. Incubation temperature affects growth and energy metabolism in blue tit nestlings. Am Nat 178:639–651.

Nord A., M.I. Sandell, and J.-Å. Nilsson. 2010. Female zebra finches compromise clutch temperature in energetically demanding incubation conditions. Funct Ecol 24:1031–1036.

Olson C.R., C.M. Vleck, and D. Vleck. 2006. Periodic cooling of bird eggs reduces embryonic growth efficiency. Physiol Biochem Zool 79:927–936.

Parker S.L. and R.M. Andrews. 2007. Incubation temperature and phenotypic traits of *Sceloporus undulatus*: implications for the northern limits of distribution. Oecologia 151:218–231.

Pieau C., M. Dorizzi, and N. Richard-Mercier. 1999. Temperature-dependent sex determination and gonadal differentiation in reptiles. Cell Mol Life Sci 55:887–900.

Schmidt K.L., E.A. Macdougall-Shackleton, and S.A. Macdougall-Shackleton. 2012. Developmental stress has sex-specific effects on nestling growth and adult metabolic rates but no effect on adult body size or body composition in song sparrows. J Exp Biol 215:3207–3217.

Shine R. 1999. Why is sex determined by nest temperature in many reptiles? Trends Ecol Evol 14:186–189.

Soderstrom K., W. Qin, and M.H. Leggett. 2007. A minimally invasive procedure for sexing young zebra finches. J Neurosci Methods 164:116–119.

Swanson D., Y. Zhang, and M. King. 2014. Mechanistic drivers of flexibility in summit metabolic rates of small birds. PLOS ONE 9:e101577.

Valenzuela N. 2004. Temperature-dependent sex determination. Pp. 211–227 in D.C. Deeming, ed. Reptilian incubation: environment and behaviour. Nottingham University Press, Nottingham.

Verhulst S., M.-J. Holveck, and K. Riebel. 2006. Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. Biol Lett 2:478–480.

Wada H., S.E. DuRant, and F.M.A. McNabb. 2018. Altricial and precocial development in birds. Pp. 50–56 in M.K. Skinner, ed. Encyclopedia of reproduction. Vol. 6. Academic Press, New York.

Wada H., B. Kriengwatana, N. Allen, K.L. Schmidt, K.K. Soma, and S.A. MacDougall-Shackleton. 2015. Transient and permanent effects of suboptimal incubation temperatures on growth, metabolic rate, immune function and adrenocortical responses in zebra finches. J Exp Biol 218:2847–2855.

Wada H., B.P. Kriengwatana, T.D. Steury, and S.A. MacDougall-Shackleton. Forthcoming. Incubation temperature influences sex ratio and offspring's body composition in zebra finches (*Taeniopygia guttata*). Can J Zool.

Warner D.A. and R. Shine. 2008. The adaptive significance of temperature-dependent sex determination in a reptile. Nature 451:566–568.

Zann R.A. 1996. The zebra finch: a synthesis of field and laboratory studies. Oxford University Press, New York.

———. 1999. Information constraints in the timing of reproduction in arid-zone birds. Pp. 265–278 in N.J. Adams and R.H. Slotow, eds. Proceedings of the 22nd International Ornithological Congress, Birdlife South Africa, Durban.

Zann R.A., S.R. Morton, K.R. Jones, and N.T. Burley. 1995. The timing of breeding by zebra finches in relation to rainfall in central Australia. Emu 95:208–222.

Zann R.A. and M. Rossetto. 1991. Zebra finch incubation: brood patch, egg temperature and thermal properties of the nest. Emu 91:107–120.

Zhao B., Y. Chen, H.-L. Lu, Z.-G. Zeng, and W.-G. Du. 2015. Latitudinal differences in temperature effects on the embryonic development and hatchling phenotypes of the Asian yellow pond turtle, *Mauremys mutica*. Biol J Linn Soc 114:35–43.