Influence of incubation recess patterns on incubation period and hatchling traits in wood ducks *Aix sponsa*

A. W. Carter, W. A. Hopkins, I. T. Moore and S. E. DuRant

A. W. Carter (afwilso@ilstu.edu) and W. A. Hopkins, Dept of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA 24061, USA. – AWC also at: School of Biological Sciences, Illinois State Univ., Normal, IL 61790, USA. – I. T. Moore, Dept of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. – S. E. DuRant, Dept of Zoology, Oklahoma State Univ., Stillwater, OK 74078, USA.

Parental effects are influential sources of phenotypic variation in offspring. Incubation temperature in birds, which is largely driven by parental behavior and physiology, affects a suite of phenotypic traits in offspring including growth, immune function, stress endocrinology, and sex ratios. The importance of average incubation temperature on offspring phenotype has recently been described in birds, but parental incubation behaviors like the duration and frequency of recesses from the nest can be variable. There are few studies describing how or if thermal variation as a result of variable incubation affects offspring phenotype. We incubated wood duck *Aix sponsa* eggs under three different incubation regimes, based on patterns that occur in nature, which varied in off-bout duration and/or temperature. We measured incubation period, morphometrics at hatching, and monitored growth and body condition for nine days post hatch. When average incubation temperature was allowed to drop from 35.9°C to 35.5°C as a result of doubled off-bout duration, we found a significant 2 d extension in incubation period, but no effects on duckling hatch mass, or growth and body condition up to nine days post hatch. However, when average incubation temperatures were equivalent (35.9°C), doubling the duration of the simulated off-bouts did not influence incubation period or any post hatch parameters. Our results suggest that if incubating parents can maintain favorable thermal environments in the nest via altered behavior (e.g. manipulating nest insulation) and/or physiology (e.g. heat production), parents may be able to avoid the costs of longer incubation periods resulting from increased off-bout duration.

Parental effects, which are causal influences of parental phenotype on offspring phenotype, are influential sources of phenotypic change (Badyaev 2008). In birds, the behavior and physiology of the incubating parents result in potent parental effects because they directly influence incubation temperature, which influences the early developmental environment and important phenotypic characteristics of offspring (Deeming 2002, Goth and Booth 2005, Olson et al. 2006, Nord and Nilsson 2012, DuRant et al. 2013b). Variability in incubation behaviors like recess duration can alter average nest temperatures and the degree of thermal constancy experienced by the embryos, which can influence important pre-hatch processes like metabolism, developmental rate, and incubation period, as well as important post-hatch traits like growth, immune function, locomotion, stress endocrinology, thermoregulation, and sex ratios (Boersma 1982, Feast et al. 1998, Goth and Booth 2005, Olson et al. 2006, DuRant et al. 2010, 2012a, b, Hopkins et al. 2011, Martin et al. 2011, Nord and Nilsson 2011, Arriero et al. 2013).

In birds, nesting behavior of incubating adults can vary widely and is influenced by the parents’ environment (Martin et al. 2007). Inexperienced foragers, or individuals nesting in resource-limited habitats or in areas of high nest disturbance may have a higher frequency of nest absence (Berrow et al. 2000, Duncan et al. 2006, Londoño et al. 2008). Nest attentiveness can also be influenced by the participation of one or both parents in incubation, the nutritional requirements of the incubator(s), cooperation between parents (e.g. males feeding incubating females), the stage of embryonic development, and climatic conditions (Skutch 1962, White and Kinney 1974, Martin and Ghalambor 1999, Conway and Martin 2000, Auer et al. 2007, Rompre and Robinson 2008). For example, silvereyes *Zosterops lateralis* increased nest attentiveness in response to artificial food supplementation (Barnett and Briskie 2010; see also Chalfoun and Martin 2007).

In addition to incubation behavior, adult physiology is an important contributor to the overall thermal environment of the nest (Deeming 2002). An incubating adult’s physiological ability to heat eggs, the size or degree of vascularization of an adult’s brood patch, and the sex of the incubator may influence the re-heating rates of eggs after a recess (Deeming 2002). For example, male chestnut-vented tit-babblers *Parsoma subcaeruleum* which lack a brood patch but devote the same amount of time to incubation as females,
maintained egg temperatures 0.3 ± 0.1°C higher than females, likely due to metabolic differences (Auer et al. 2007, but see Kleindorfer et al. 1995).

Behavioral and physiological parental effects during incubation not only influence average incubation temperature, but also determine the frequency and degree of temperature fluctuation experienced by the embryos. Martin et al. (2007) found that nest temperatures fluctuated more frequently during the day when nest attentiveness was more variable (see also Martin et al. 2013). In general, the longer an adult spends off of the nest the cooler the eggs become (Calder III 2002). However, the rate of cooling is highly dependent upon the size of the egg, clutch size, developmental stage of the embryos, ambient air temperature, and the degree and type of insulation in the nest (White and Kinney 1974, Calder III 2002, Turner 2002, Hilton et al. 2004). As egg temperature decreases in response to parental absence, the amount of energy to reheat the eggs increases as does the amount of time required to return eggs to a temperature favorable for embryonic development (Biebach 1986, Calder III 2002, Olson et al. 2006). While previous research has demonstrated the influence of variable thermal environments on offspring phenotype in reptiles (and birds to a lesser degree), no studies have concomitantly examined how the interplay between incubation constancy and average incubation temperature influences avian phenotype (Les et al. 2009, Paitz et al. 2010).

We investigated the influence of female recess behavior on incubation period and offspring phenotype by experimentally incubating wood duck Aix sponsa eggs under three different regimes. Specifically, we evaluated whether extended simulated recesses that resulted in either decreased average incubation temperature or maintenance of the average incubation temperature, would influence incubation period, hatching success, early survival, growth, and body condition of ducklings. Our prior research on wood ducks demonstrated that <1°C difference in average incubation temperature has significant effects on wood duck duckling phenotype (DuRant et al. 2010, 2012a, b, Hopkins et al. 2011). We predicted that ducklings from eggs incubated with the longest simulated recesses and lowest average incubation temperature would have longer incubation periods and lower survival and growth than ducklings from eggs incubated with shorter simulated recesses and higher average temperatures.

**Material and methods**

**Study species**

The wood duck is a dabbling duck that inhabits wooded margins of rivers, streams, and lakes, and begin nesting as early as late January (Hepp et al. 1987, Bellrose et al. 1994). Wood ducks nest primarily in abandoned woodpecker cavities, but will also exploit artificial nest boxes (Hepp et al. 1987, Bellrose et al. 1994). Their average clutch size is 12 eggs, with one egg laid per day and the incubation period ranges from 28–37 d in length (Hepp et al. 1987, Bellrose et al. 1994). Wood ducks are gyneparental (female-only incubation) and nesting behavior is variable and contributes to variation in incubation temperature and period (Manlove and Hepp 2000). In particular, female recesses from the nest show a wide range of temporal variability (Bellrose et al. 1994). In our population of wood ducks, females most commonly (73.8%) take two recesses per day, in the morning and the evening, with recess durations ranging from 42.5 to 169.2 min each (Manlove and Hepp 2000). Average incubation temperatures in our population range from 34.8–37.8°C with 36.2 ± 0.4°C occurring most commonly. McClintock (unpubl. Ms thesis 2013) examined egg cooling rates in the same population with varying ambient temperatures and amounts of insulation. She found that eggs exposed to 22°C with 0.5 g of feather insulation cooled from 37°C to ~29°C in two h, which resembles the conditions simulated in the recess regimes utilized in our current study.

**Study population**

Wood duck eggs were collected in early summer 2012 from nest boxes on a large reservoir (L-lake) and five ephemeral wetlands (n = 28 boxes total), located on the Dept of Energy’s Savannah River Site in South Carolina, USA (33.1°N, 81.3°W). Nest boxes at these sites have been used by wood ducks for >25 yr and have been monitored by researchers every year. Egg incubation, hatching, animal husbandry and experiments were conducted in a laboratory at Virginia Tech using approved IACUC procedures (no. 12-045-FIW).

**Egg collection and incubation**

Once nesting began, nest boxes were checked daily and freshly laid eggs were individually marked and stored at 20°C and 55–60% humidity until they were placed in incubators (2–5 d after collected; holding eggs up to 10 d does not affect egg viability, Walls et al. 2011). To account for clutch effects, eggs from a common clutch were dispersed across the 3 incubation treatments. Treatments were designed to fall within the natural range of average incubation temperatures (34.8–37.8°C; Hepp et al. 2006) and recess patterns (Bellrose et al. 1994) of wood ducks in the wild. All incubation regimes remained above 26°C, a lower thermal limit to avian embryonic development where development is greatly reduced and energy is lost through respiration (Webb 1987). We used an incubation pattern commonly observed in the field that in previous research produced viable offspring in the lab and field as our reference incubation treatment (Hepp et al. 2006, DuRant et al. 2010, 2011, 2012a, b). Eggs were incubated in Grumbach incubators which maintained humidity levels between 60–65%. Incubator temperature and humidity were continuously monitored with data loggers. Eggs were candled regularly to monitor development and embryonic survival. Days to pipping and hatching were recorded for each individual.

The three incubation treatments were as follows (Fig. 1): 1) a common incubation pattern. This treatment maintained an overall average temperature of 35.9°C with the most common recess pattern observed in wild ducks of two
temperatures ranged from 36.6 °C during simulated incubation to a low of 30.1 °C during simulated recesses. Treatment 1 maintains an optimal average incubation temperature of 35.9 °C and typical temporal recess pattern that includes two 60 min recesses per day. Treatment 2 simulates a female compensating physiologically or behaviorally (higher active incubation temperature) for increased recess durations of 120 min resulting in maintenance of the warmer average temperature. Treatment 3 simulates a female that does not compensate for extended recess durations of 120 min, and thus has a lower average incubation temperature of 35.5 °C.

Duckling husbandry

After hatching, ducklings were housed in 46 × 32 × 24.5 cm plastic cages, arranged in a rack system within an environmental chamber kept at 28 °C. Each cage had a heat lamp (50 W) suspended above one end of the cage to supply additional heat. Ducklings were housed in pairs, fed ad libitum and allowed continuous access to water. Cages and dishes were cleaned each morning.

We measured body mass (g), tarsus length (mm), and culmen length (mm) for each individual upon hatching (n = 58; treatment 1 n = 17; 2 n = 18; 3 n = 23; from a total of 20 clutches). We measured duckling mass and tarsus each morning to nine days post hatch, and monitored survival every morning and afternoon.

Statistics

All statistical analyses were performed in SAS ver. 9.1 and the threshold for statistical significance was set at α < 0.05. Where appropriate, we tested for normal distribution and homoscedasticity using Ryan – Joiners and Levene – Bartlett’s tests, respectively. Hatching success (full emergence from egg) was compared among the three incubation treatments using a generalized linear mixed model (SAS proc glimmix) with female included as a random effect. Since post-hatch survival of ducklings was high, we analyzed the survival data using a Fisher’s exact test (SAS proc freq). We analyzed the effect of incubation treatment on incubation period with a one-way ANOVA (SAS proc glm) as well as individual repeated measures ANOVAs (SAS proc glimmix) with female included as a random effect. We analyzed all growth measurements (mass, tarsus, body condition (calculated as residuals of body mass/tarsus length)) with a repeated measures MANOVA (SAS proc glm) as well as individual repeated measures ANOVAs (SAS proc glm) with female included as a random effect. To account for clutch effects on growth and condition analyses, eggs from the same clutch were spread across treatments. In cases where more than one egg per clutch was allocated to a treatment, pseudoreplicates (i.e. siblings) within each treatment were averaged and analyzed as a single value.

Results

Hatching success on average (overall average = 60%) was low compared to previous studies in our lab (DuRant et al. 2010, 2012a, b), but did not significantly vary among incubation treatments (incubation treatment 1: 53%; 2: 58%; and 3: 70%; F2,31 = 0.80, p = 0.46). Egg storage time before being set in the incubator did not differ among treatments (F1,55 = 1.08, p = 0.348; mean days to set: treatment 1: 2.6; treatment 2: 2.5; treatment 3: 3.1) and did not influence incubation period (F1,49 = 0.65, p = 0.423) and was therefore left out of the models. However, incubation treatment significantly influenced incubation period (F2,35 = 41.17, p < 0.001) with eggs from the third incubation treatment (extended recesses without thermal compensation) exhibiting a two day longer incubation period than the other two experimental treatments (Tukey HSD; post hoc analysis: 1 × 2 p = 0.78, 1 × 3 p < 0.001, 2 × 3 p < 0.001) (Fig. 2). Post-hatch survival to day 9

Figure 1. A graphical display of incubation temperature throughout a 24 h period in each treatment. (A) shows a typical 24 h incubation cycle with two simulated off-bouts. (B) shows a single simulated off-bout. Treatment 1 maintains an optimal average incubation temperature of 35.9 °C and typical temporal recess pattern that includes two 60 min recesses per day. Treatment 2 simulates a female compensating physiologically or behaviorally (higher active incubation temperature) for increased recess durations of 120 min resulting in maintenance of the warmer average temperature. Treatment 3 simulates a female that does not compensate for extended recess durations of 120 min, and thus has a lower average incubation temperature of 35.5 °C.
was high on average (93%) and did not differ among incubation treatments (p = 0.37; treatment 1: 100%, 2: 94%, 3: 87%). Incubation treatment did not significantly influence mass, tarsus or body condition at hatching (0 dph) (one-way ANOVA: mass $F_{2,25} = 0.94$, $p = 0.40$; tarsus $F_{2,25} = 1.37$, $p = 0.27$; body condition $F_{2,25} = 1.32$, $p = 0.28$). All ducklings grew in mass and tarsus length, and increased in body condition over the course of the study period (repeated measures ANOVA: age: mass $F_{9,421} = 431.27$, $p < 0.0001$; tarsus $F_{9,421} = 238.77$, $p < 0.0001$; body condition $F_{9,421} = 20.0$, $p < 0.0001$). However, incubation treatment did not influence these growth parameters (repeated measures ANOVA; in all cases $p > 0.21$, Fig. 3).

Discussion

Our study demonstrated that if an incubating parent can physiologically and/or behaviorally maintain a higher active incubation temperature (by $< 0.5\, ^\circ\text{C}$), the parent can compensate for doubling total off-bout duration from the nest and maintain an ideal average egg temperature to avoid the costs associated with an extended incubation period (e.g. increased predation risk or time and energy investment). These findings agree well with previous work (Martin et al. 2007). Contrary to our predictions, we did not find any effect of incubation treatment on hatch mass, survival, growth, or body condition. However, the incubation treatment that simulated temperature compensation by the incubating parent resulted in a shorter incubation period (two days) than the incubation treatment without simulated temperature compensation. Our incubation conditions represent the common recess patterns observed in our population of wood ducks, rather than the most extreme combination of recess frequency and duration. As such, our results may underestimate the effects that more extreme recess conditions may have on offspring phenotype.

The effect of recess duration on incubation period alone has the potential to significantly influence adult physiology and survival and hatchling success early in development (Spear and Nur 1994, Tombre and Erikstad 1996). Incubation is an energetically costly component of avian parental care and extended incubation periods can increase energetic investments of parents (Biebach 1986, Williams 1993, Deeming 2002, DuRant et al. 2013a). Incubation period is also an early determinant of offspring success. Longer incubation periods leave nests exposed to predators for a longer duration suggesting fitness advantages to shorter incubation periods (Martin 2002, Hepp et al. 2006). For example, barnacle goose nests with experimentally longer incubation periods lost more eggs to predation than nests with shorter incubation periods (Tombre and Erikstad 1996). In addition, there are physiological costs incurred by offspring with longer incubation periods. DuRant et al. (2011) found that ducklings incubated at lower temperatures resulting in longer incubation periods (by $\sim 5$ d) expended 20–37% more energy during embryonic development and hatching than ducklings incubated at lower temperatures resulting in longer incubation periods (by $\sim 5$ d) expended 20–37% more energy during embryonic development and hatching than ducklings incubated at...
warmer temperatures with shorter incubation periods. Lastly, there may be competitive advantages for individuals that hatch before the rest of their cohort. In the present study, wood duck ducklings across all treatments increased in body mass by 13–23% in two days. This underscores the potential competitive advantage of a two days head start that older ducklings from earlier hatched clutches may gain when compared to ducklings that hatch later.

There are many strategies that incubating birds employ to regulate incubation temperature both physiologically and behaviorally and these strategies could be utilized to thermally compensate for increased off-bout duration. Females can increase the amount of heat delivered to the eggs via the brood patch after a recess by using heat collected from activity during the off-bout, or increasing production of metabolic heat (Biebach 1986, Deeming 2002). Studies that have artificially cooled eggs demonstrated higher breathing rates of incubating females (Haftorn and Reinertsen 1982, 1985), higher oxygen consumption (Vleck 1981), increased heart rate (Gabrielsen and Steen 1979), shivering (Toien et al. 1986), and vasodilation and increased blood flow to the brood patch (Midtgård et al. 1985). In addition to physiologically managing how much heat is delivered to the eggs, incubating birds can also behaviorally regulate the temperature experienced by the embryos which may act as another mechanism that females could thermally compensate for increased off-bouts. Anseriformes pluck feathers from their breast exposing a brood patch that assists in the distribution of heat to the eggs, while also incorporating feathers to the construction of the nest to aid in insulation which may reduce the energy required to reheat eggs after a recess (Lea and Klandorf 2002, Hilton et al. 2004). In an experimental manipulation, McClintock (unpubl. MS thesis 2013) found that the amount of insulation (0.5 vs 4.0 g) in wood duck nests significantly influenced morning recess duration late in incubation, suggesting that females modulate their incubation behavior in response to nest conditions, which in turn influences incubation constancy experienced by developing embryos.

While our study did not result in phenotypic differences among offspring incubated with extended recess durations, previous research in amniotic reptiles and birds has demonstrated a clear effect of thermal variability on embryonic and hatching characteristics. Incubation temperature fluctuations about a common mean temperature in amniotic reptiles can be influential in determining offspring phenotype including morphology and sex (Les et al. 2009, Paitz et al. 2010). In birds, Feast et al. (1998) found that short-term (36–24 h) reductions in incubation temperature to 22°C from 37.5°C decreased growth rates and yolk lipid uptake by the liver in chicken embryos. Moreover, Olson et al. (2006) reported decreased growth rates, reduced yolk reserves, and higher mass-specific metabolic demands in embryonic zebra finches Taeniopygia guttata incubated at 37.5°C with 15 1-h periods of 20°C each day. A fourth incubation regime with the same average temperature as treatment 3 (35.5°C) but 2 60-min recesses would have been an ideal additional treatment. Constrained by the number of incubators available, however, we selected the combination of regimes that best addressed the possibility of female thermal compensation.

The ability to thermally compensate for increased off-bouts may allow avian parents’ to offset unfavorable nesting conditions. For example, parents nesting in areas of high disturbance from predators or humans that result in longer off-bouts may be able to mitigate consequences on incubation period, if they are still able to maintain optimal temperatures. Future studies could experimentally manipulate parent off-bout frequency or duration (Reid et al. 2000, Hanssen et al. 2003, McClintock unpubl. MS thesis 2013) and quantify the effects on incubation temperature and subsequent effects on offspring phenotype. Future research also is needed to quantify the physiological and behavioral limits to thermal compensation during incubation. Lastly, our study has implications for avian nesting biology in the face of climate change where more frequent or severe periods of inclement weather are predicted. Plasticity in incubation temperature regulation may aid parents’ ability to incubate eggs at temperatures conducive to embryonic development during inclement weather when parents must balance the concomitant energy demands of incubation and maintenance (Nord et al. 2010).

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