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Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird

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Summary

1. The developmental environment plays a key role in determining offspring phenotype, and the parents' behaviour and physiology often dictates developmental conditions. Despite the plethora of studies documenting the importance of incubation temperature on offspring phenotype in reptiles, very few studies have examined such relationships in birds.

2. Because nearly all birds physically incubate their eggs, altering the nest environment may be an important but previously overlooked way parents can influence their offspring's phenotype. Here, we tested the hypothesis that incubation temperature would affect thermoregulation in wood duck (*Aix sponsa*) hatchlings.

3. We show that a reduction in < 1 °C in incubation temperature affects the metabolic costs of thermoregulation in offspring of a non-domesticated bird, resulting in 27–40% greater increases in oxygen consumption of ducklings incubated at the lowest temperature relative to ducklings incubated at higher temperatures.

4. Because we demonstrate that incubation temperature affects hatchling phenotypic quality, our findings provide novel support for newly proposed frameworks that highlight the importance of incubation temperature to the evolution of clutch size in birds.

Key-words: *Aix sponsa*, bioenergetics, endothermy, incubation temperature, maternal effects, wood duck

Introduction

Avian parents devote tremendous effort towards rearing their young. Because of the high demands of reproduction in birds, parents are constrained in the number of young they can successfully produce in a single nesting attempt (Lack 1947; Stearns 1992). For precocial species, Lack (1967, 1968) proposed that one of the primary constraints on clutch size is the amount of food available to laying females. More recent research, however, suggests that females may also be constrained in the number of eggs they can successfully incubate. Studies have shown that incubating larger clutches can come at significant costs, with larger clutches reducing future survival and reproduction of incubating parents (Hanssen *et al.* 2005; de Heij, van den Hout & Tinbergen 2006), requiring longer incubation periods (Larsen, Lislevand & Byrkjedal 2003) and producing young of lower quality (Larsen, Lislevand & Byrkjedal 2003). Incubation temperature in particular can vary with clutch size (Cooper *et al.* 2005), and differences in temperature have been shown to affect offspring phenotype (Hepp, Kennamer & Johnson 2006; Ardia, Perez & Clotfelter 2010; DuRant *et al.* 2010, 2011). Because incubation conditions can affect offspring phenotype and potentially influence fitness of the young, incubation costs may have contributed importantly to the evolution of clutch size in birds.

One of the most influential periods for phenotypic expression is during early development when key physiological and neurological systems are forming. The behaviour and physiology of parents can have tremendous influence on the early developmental environment of their young, which helps to shape their offspring's phenotype via non-genomic contributions (i.e. parental effects; Badyaev & Uller 2009). Because birds physically incubate their eggs, brooding behaviour provides a clear connection between avian parental behaviour and incubation conditions experienced by the embryos. Physical contact with the eggs influences important aspects of embryonic microclimate including nest temperature and humidity (Deeming 2002; Martin *et al.* 2007). Recent research in non-domesticated birds demonstrates that incubation conditions can affect a suite of phenotypic traits in hatchling birds, many of which have implications for future development and survival (Cyr & Romero 2007; Ardia, Perez & Clotfelter 2010). In fact, a series of recent laboratory

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studies pinpoint incubation temperature as a major contributor to such phenotypic variation. These studies demonstrate that small differences in incubation temperature, reflecting variation in temperature found in naturally incubated nests, drastically affect hatchling immunocompetence (DuRant *et al*. 2011), locomotor performance (Hopkins *et al*. 2011), stress endocrinology (DuRant *et al*. 2010), growth, and body condition (DuRant *et al*. 2010).

For precocial species, perhaps one of the most important traits influencing the survival of hatchlings is the early development of thermoregulatory ability. Among some waterfowl, mortality of hatchlings due to hypothermia is estimated to be as high as 25% (Korschgen *et al*. 1996). As hatchling birds transition to homeothermy, they rely on energy and protein stores and frequent brooding to survive cold periods. Development of homeothermy requires maturation of integument and increased protein in skeletal muscles (Visser1998). A previous study in wood ducks (*Aix sponsa*; Fig. 1) revealed that just prior to hatching (star pip, ~1–3 day prior to full hatching) ducklings from eggs incubated at lower temperatures had lower protein content than ducklings incubated at higher temperatures (Hepp, Kennamer & Johnson 2006). In addition, ducklings incubated at lower temperatures expended more energy, as noted by higher oxygen consumption, during incubation than ducklings from higher incubation temperatures (DuRant, Hopkins & Hepp 2011). These studies suggest that ducklings incubated at lower temperatures may have fewer energy reserves remaining after hatching to meet the demands of thermoregulatory challenges than ducklings from higher incubation temperatures. Because wood ducks begin nesting in late winter, they are frequently exposed to low temperatures (at times below freezing) that pose substantial physiological challenges for ducklings.

In this study, we tested the hypothesis that incubation temperature affects thermoregulation in hatchling wood ducks, predicting that ducklings hatched from eggs incubated at lower temperatures would be less effective and efficient at maintaining their body temperatures. To eliminate other environmental factors that affect avian development, we incubated wood duck eggs in the laboratory at three temperatures (35·0, 35·9 and 37·0 °C) that fall within the range of temperatures of naturally incubated nests (range 34·8–37·8 °C; Hepp, Kennamer & Johnson 2006). Twenty-four hours after hatching, we tested ducklings in one of two thermal challenge experiments, following the methods similar to those of Rhymer (1988). In the first experiment, we measured the change in a ducklings' body temperature after being exposed to a 1-h thermal challenge at 5, 10, 15, 20 or 36 °C (controls). In the second experiment, we estimated energy expenditure of ducklings for 1 h at 36 °C, and then again during a 1-h thermal challenge at 15 °C. We indirectly measured energy expenditure by monitoring duckling oxygen consumption (Dorcas, Hopkins & Roe 2004; Hopkins *et al*. 2004), which can be converted into energy equivalents assuming 1 L O_2 = 19·6 kJ (Vleck, Vleck & Hoyt 1980).

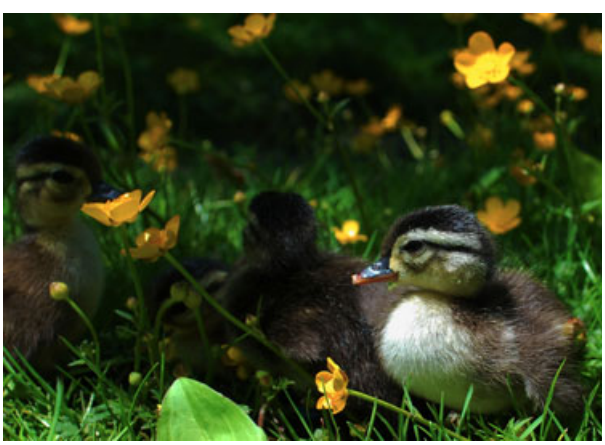

Fig. 1. One day post-hatch wood duck (*Aix sponsa*) ducklings.

Materials and methods

EGG COLLECTION AND INCUBATION

Every 4 days until egg laying was initiated, we checked wood duck nest boxes (n = 201) located on two large reservoirs and several isolated wetlands in west-central South Carolina. We visited active nests daily and collected and marked any new eggs. Eggs were stored at 20 °C and 55–60% humidity. After 4 days of collection, we transported eggs to Virginia Tech and artificially incubated them in Grumbach incubators (model BSS 160; Munchholzhausen, Germany) at one of three temperatures (35·0, 35·9 and 37·0 °C) at 60–65% humidity, which produced three incubation durations [37·2 ± 0·24, 34·4 ± 0·18 and 32·1 ± 0·18 days, respectively). Incubators were not maintained at constant temperatures but were programmed for two cool-down periods each day (~3 °C reduction in mean temperature for 75 min at 0815 and 1830 h) to simulate natural daily feeding recesses taken by mothers during incubation (Manlove & Hepp 2000). All experimental procedures were conducted in accordance with approved Virginia Tech IACUC protocols.

DUCKLING HATCHING AND DUCKLING HUSBANDRY

We monitored hatching success of eggs incubated at the three temperatures by checking for newly hatched ducklings four times a day between 08.00 and 20.00 h. Upon hatching, we measured duckling mass and tarsus length and calculated body condition as the residual of mass regressed against tarsus. We maintained ducklings communally in 46 × 32 × 24·5 cm plastic cages (2–3 ducklings/cage) in a temperature-controlled environmental chamber (28 °C, 14 h:10 h light/dark photoperiod). A 50-watt infrared light bulb suspended above each cage provided additional warmth to ducklings, creating a thermal gradient of 30–37 °C. Ducklings were allowed constant access to water.

THERMOREGULATION AND BIOENERGETICS OF DUCKLINGS

We examined the thermoregulatory capacity and energetics of ducklings by conducting cold-challenge experiments similar to those described by Rhymer (1988). In the first experiment, unfed 1-day-old ducklings previously incubated at one of three experimental temperatures (35·0, 35·9 and 37·0 °C) were placed in individual 1-L glass

chambers within an environmental chamber and allowed to acclimate for 1 h at 36 °C (n = 1–8 ducklings measured per trial). Next, we measured body temperature of each bird using a cloacal thermometer (Scultheis T6000; Miller and Weber Inc, New York, NY, USA), then dropped temperatures of the environmental chambers to 5, 10, 15 and 20 °C, or left the temperature at 36 °C (these ducklings served as controls) for 1 h (n = 11–33 ducklings/per incubation temperature/per challenge temperature). We chose thermal challenge temperatures that we believed would fall below the thermoneutral zone of young wood ducks. Although the thermoneutral zone of wood duck ducklings is not known, the lower critical temperature of other dabbling ducks such as mallard (*Anas platyrynchos*) and Eurasian teal (*Anas crecca*) at 1 dph is 32 °C (Koskimies & Lahti 1964). After 1 h at the ducklings' thermal challenge temperature, we again measured duckling body temperature. We used the percent change in a ducklings' body temperature from before to after the thermal challenge in statistical analyses. All of our challenge temperatures were within the range of temperatures experienced by ducklings in the field in South Carolina, USA.

Our second challenge experiment examined the metabolic costs associated with maintaining homeothermy. An unfed 1-day-old duckling that hatched from an egg previously incubated at one of the three experimental temperatures (35·0, 35·9 and 37·0 °C; n = 10–12 ducklings/per incubation temperature) was placed in a 1-L container and held in an environmental chamber at 36·0 °C (approximating thermoneutrality). After allowing ducklings (n = 1–4 ducklings per trial) to settle for 1 h, we measured duckling respiration every 12 min for 1 h using open flow respirometry (MicroOxymax; Columbus Instruments, Columbus, OH, USA). We circulated excurrent air drawn from each respiratory chamber through a hygroscopic Nafion tube drier (Columbus Instruments) to remove water vapour before determining oxygen consumption rates using an electrochemical fuel cell. We simultaneously determined CO_2 production rates in each chamber, and oxygen consumption values were corrected for CO_2 concentrations using the MICROOXYMAX software. Additional details on respirometry techniques are provided in the study of Dorcas, Hopkins & Roe (2004) and Hopkins *et al.* (2004). Ducklings exhibited little activity during this hour as noted by their stable metabolic profiles. We then measured each duckling's body temperature using a cloacal thermometer in < 2 min to minimize disturbance and potential influences on metabolic rate. Next, we dropped the environmental chamber to 15 °C over a 15-min period and monitored respiration for an additional 1 h. At the end of the 1-h thermal challenge, we again measured duckling body temperature. To try to minimize the confounding influence of body mass on respiration, we used ducklings within a more narrow range of body sizes in this experiment. We obtained five respiration measurements for our estimate of pre-trial respiration (or resting metabolic rate; RMR) and five measurements for our estimate of respiration during the thermal challenge. We calculated the integral of the respiration curves at thermoneutral (36 °C) and 15 °C to determine the volume of oxygen consumed before the thermal challenge and during the thermal challenge. Integrals were compared to determine the relative cost of homeothermy.

To avoid pseudoreplication and account for parental effects on duckling thermoregulation and respiration, we only used one duckling per clutch per incubation temperature × thermal temperature treatment in both experiment 1 and 2.

STATISTICAL ANALYSES

All statistical analyses were run in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) or Microsoft Excel, and statistical significance was recognized at $\alpha < 0{\cdot}05$. Where appropriate, we tested for normal distribution of the data and homoscedasticity using Ryan–Joiners and Bartlett's tests, respectively. Unless otherwise noted, raw data were used in statistical analyses.

Results

There was no difference in fresh egg mass among incubation temperatures (ANOVA; SAS Proc GLM: P = 0·178). Average egg mass prior to incubation at each temperature was as follows: (means ± 1 SE) 35·0 °C = 40·4 ± 0·3, 35·9 °C = 41·1 ± 0·3 and 37·0 °C = 41·0 ± 0·2. Using a Chi-square test (SAS proc freq), we determined that hatching success did not differ among incubation temperatures (P = 0·189; 35·0 °C = 63%, 35·9 °C = 71% and 37·0 °C = 70%). However, the length of the incubation period differed significantly among incubation temperatures, with lower temperatures slowing developmental rates (ANOVA; SAS Proc GLM; $F_{2,\ 342}$ = 359·2; $P < 0{\cdot}001$; see methods for incubation durations). *Post hoc* comparisons (Tukey HSD) revealed that all incubation temperatures produced incubation periods that differed from one another.

To test for the effects of incubation temperature on duckling thermoregulatory ability, we used an ANCOVA (SAS Proc Mixed). Models were performed with both hatchling mass and body condition as covariates, but because hatchling mass had a stronger influence on changes in body temperature, we used mass as the covariate in our final model. As the challenge temperature decreased, duckling body temperature also decreased (thermal challenge temperature: $F_{4,\ 278}$ = 22·26; $P < 0{\cdot}001$; Fig. 2). However, there was no effect of incubation temperature on changes in a duckling's body temperature (incubation temperature: P = 0·930; incubation

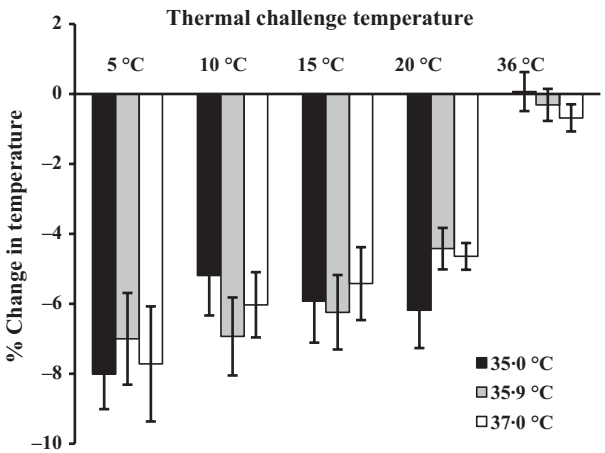

Fig. 2. The effect of incubation temperature on thermoregulatory ability of hatchling wood ducks (*Aix sponsa*). Means (±1 standard error) represent the percent change in a duckling's body temperature after being held at thermoneutral (36 °C) for 1 h then being thermally challenged for 1 h at one of five temperatures (5, 10, 15, 20 or 36 °C). Ducklings hatched from eggs incubated at one of three temperatures (35·0, 35·9 or 37·0 °C). n = 11–33 ducklings/thermal challenge temperature/incubation temperature.

temperature × thermal challenge temperature: $P = 0{\cdot}499$). Post hoc comparisons (Tukey HSD) revealed that the percent change in body temperature of ducklings challenged at 5, 10, 15 and 20 °C was greater than ducklings held at 36 °C (control ducklings). We also detected a significant effect of mass on changes in a ducklings body temperature where smaller ducklings were less effective at maintaining their body temperature than larger ducklings (hatchling mass [covariate]: $F_{1,\ 278} = 6{\cdot}45$; $P = 0{\cdot}012$). We detected a similar trend, although not statistically significant, when we included body condition as a covariate in the model; ducklings in poorer body condition exhibited the greatest decreases in body temperature (body condition: $F_{1,\ 278} = 2{\cdot}91$; $P = 0{\cdot}089$).

To determine whether hatchling mass and body condition of ducklings used in experiment 1 differed among incubation temperature treatments, we used ANCOVA (SAS Proc GLM) with egg mass included as the covariate. Body condition of ducklings used in experiment 1 differed significantly among incubation temperatures ($F_{2,\ 290} = 6{\cdot}09$; $P = 0{\cdot}003$), with the low incubation treatment producing ducklings in the poorest body condition (mean body condition residual ± 1 SE: 35·0 °C: −1·0 ± 0·3; 35·9 °C: 0·2 ± 0·3; 37·0 °C: 0·4 ± 0·2). There was a similar trend, although not statistically significant, with hatchling mass ($F_{2,\ 291} = 2{\cdot}37$; $P = 0{\cdot}095$); the lowest incubation temperature produced less heavy ducklings than the higher incubation temperatures (mean mass ± 1 SE: 35·0 °C: 25·0 ± 0·3; 35·9 °C: 26·0 ± 0·3; 37·0 °C: 26·1 ± 0·2). Egg mass also significantly affected hatchling mass and hatchling body condition (hatchling mass: $F_{1,\ 290} = 303{\cdot}05$; $P < 0{\cdot}001$; body condition: $F_{1,\ 290} = 526{\cdot}71$; $P < 0{\cdot}001$), where larger eggs produced heavier ducklings in better body condition.

Using an ANCOVA (SAS Proc Mixed) with $\log_{10}$-transformed oxygen values, we determined that although ducklings from all incubation temperatures exhibited similar changes in body temperature when confronted with a thermal challenge, ducklings that hatched from eggs incubated at the lowest temperature had a greater increase in oxygen consumption during a thermal challenge than ducklings incubated at the higher temperatures (incubation temperature × time: $F_{2,\ 31} = 5{\cdot}60$; $P = 0{\cdot}008$; Figs 3 and 4). In addition, there was a significant positive relationship between oxygen consumption and duckling body condition (body condition: $F_{1,\ 30} = 11{\cdot}66$; $P = 0{\cdot}002$) and hatchling mass (hatchling mass [covariate]: $F_{1,\ 30} = 6{\cdot}74$; $P = 0{\cdot}015$) on oxygen consumption.

Again, we tested for differences among incubation temperature treatments in hatchling mass and body condition of ducklings used in experiment 2 using ANCOVA (SAS Proc GLM) with egg mass included as the covariate. There was no difference among incubation temperatures in hatchling mass (mean mass ± 1 SE: 35·0 °C: 21·5 ± 0·2; 35·9 °C: 21·4 ± 0·5; 37·0 °C: 21·9 ± 0·7) or body condition (mean body condition residual ± 1 SE: 35·0 °C: −0·5 ± 0·2; 35·9 °C: −0·2 ± 0·5; 37·0 °C: 0·6 ± 0·4) of ducklings in experiment 2 ($P = 0{\cdot}479$ and $0{\cdot}255$, respectively). Hatchling mass and body condition were significantly affected by fresh egg mass in which larger eggs produced heavier ducklings in better body condition (hatchling mass: $F_{1,\ 30} = 61{\cdot}68$; $P < 0{\cdot}001$; body condition: $F_{1,\ 30} = 40{\cdot}54$; $P < 0{\cdot}001$).

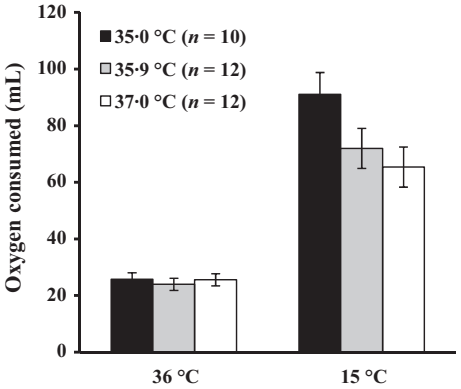

Fig. 3. The effect of incubation temperature on oxygen consumed during a thermal challenge in 1 day post-hatch wood ducks (*Aix sponsa*). Least-squares means (corrected for body mass) represent the volume of oxygen consumed (ml; integral of respiration curve) of ducklings held at thermoneutral (36 °C) for 1 h then thermally challenged at 15 °C for 1 h. Ducklings hatched from eggs incubated at one of three temperatures (35·0, 35·9 or 37·0 °C). Statistical analyses were conducted on $\log_{10}$-transformed oxygen data. Error bars are ± 1 standard error of the LSmean.

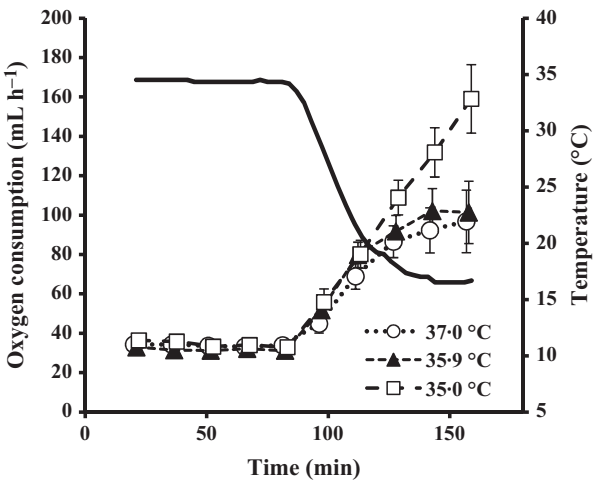

Fig. 4. Patterns of oxygen consumption (mL h^{-1}) of 1 day post-hatch wood duck (*Aix sponsa*) ducklings while at thermoneutral (36 °C) then during a 1 h thermal challenge (15 °C). Ducklings hatched from eggs incubated at one of three temperatures (35·0, 35·9 or 37·0 °C). Oxygen consumption is presented as LSmeans corrected for body mass. The solid line represents the temperature inside individual respiration chambers. Error bars are ± 1 standard error of the mean. 35·0 °C: $n = 10$; 35·9 °C: $n = 12$; 37·0 °C: $n = 12$.

Discussion

Our findings reveal that incubation temperature is an important determinant of avian offspring phenotype, a phenomenon that has rarely been considered by avian ecologists (Deeming 2004). Here, we demonstrate, for the first time, that

slight differences in incubation temperature, within the range found in naturally incubated nests, can affect energy expended by non-domesticated avian hatchlings during thermoregulation. In the wild, most young birds likely develop in environments with limited resource availability, and thus, may not be able to compensate for large increases in energy expenditure. Energetic constraints on young could ultimately have implications for traits important to survival and reproduction (e.g. immunocompetence, growth and size at maturity), suggesting that there is selection pressure on parents to maintain optimal incubation temperatures, in some cases within a very narrow range. For example, in our study population of wood ducks, incubation temperatures in the field approximate a normal distribution with only a small proportion of females producing the cooler and warmer extremes of observed nest temperatures (Hepp, Kennamer & Johnson 2006). It is interesting that in the laboratory, the cooler extreme, which females seem to avoid in the wild, also produces ducklings with poorer physiological performance relative to ducklings incubated in the mid- to warmer temperatures (DuRant *et al.* 2010, 2011; DuRant, Hopkins & Hepp 2011; Hopkins *et al.* 2011). Although not yet tested, a similar negative effect on hatchling phenotype also could be present at the warmest temperature extreme.

Contrary to our prediction, incubation temperature did not affect a duckling's ability to maintain its body temperature during a brief thermal challenge (Fig. 2). However, thermal challenge temperature did affect changes in body temperature, with all ducklings exhibiting greater decreases in body temperature as thermal challenge severity increased. Average body temperature of all ducklings at 1 day post-hatch (dph) at thermoneutral was 38·6 ± 0·05 °C, which is slightly lower than other duck species at 1 dph (e.g. Mallards, Common Goldeneye, Pekin ducks, Common Merganser: 39·0–40 °C; Koskimies & Lahti 1964). Similar to the young of other precocial species, hatchling wood ducks appear to be relatively resilient to changes in body temperature as their body temperature regularly dropped 2–4 °C when exposed to a thermal challenge (Visser 1998). During our study, mortality only occurred in ducklings (8 of 294 ducklings) whose body temperature dropped below 33 °C. Resilience to changes in body temperature is thought to allow ducklings to transition to homeothermy without constant reliance on brooding from parents or huddling with siblings (Visser 1998). After nest exodus, ducklings move to nearby water and immediately begin foraging on their own; they are typically only brooded by females during inclement weather (Bellrose & Holm 1994). Because wood ducks in South Carolina begin hatching in early March and leave the nest within 24 h, the ability to tolerate brief reductions in body temperature would increase their chances of survival during the first days after hatching.

Consistent with our predictions, incubation temperature had a strong effect on energy expended by ducklings during a thermal challenge. Despite similar RMR among incubation treatments, ducklings incubated at the lowest temperature had 27 and 40% higher metabolic rates during the thermal trial than ducklings incubated at the medium and high temperatures, respectively (Figs 3 and 4). Our results suggest that, even though ducklings incubated at the lowest temperature maintain their body temperature as well as those incubated at higher temperatures, they expend more energy to do so. Although no other studies have examined the influence of ecologically relevant differences in incubation temperature on avian thermoregulation, a series of studies on domesticated species (domestic poultry and Muscovy ducks) suggest that brief exposure to reduced temperature during late incubation can influence offspring thermoregulation (summarized in Nichelmann 2004). Together, our work and the poultry work indicate that the effects of temperatures experienced during incubation on avian thermoregulation warrant further study in wild species.

Previous studies have shown that body composition of ducklings differs among incubation temperatures with protein content decreasing with decreasing incubation temperature (Hepp, Kennamer & Johnson 2006). Lower protein composition of ducklings from the lowest incubation temperature could account for their lower thermoregulatory efficiency because protein content of the leg and pectoral muscles is important in generating heat (Visser 1998). The disparity in duckling thermoregulatory ability among incubation treatments does not appear to be driven by differences in size and surface area to volume (SA/V) ratios as there was no difference in duckling size or body condition among incubation temperatures because of the small range of duckling sizes intentionally represented in the second experiment (see methods). Moreover, incubation temperature affected metabolism during a thermal challenge even after body size and condition were accounted for in statistical models. Greater energy expenditure in ducklings incubated at the lowest temperature could arise from differences in insulation. Less insulation could increase heat loss and therefore require greater heat production to compensate for these losses. Feather density is critical for insulation in young ducklings, and future studies should quantify whether incubation temperature affects duckling feather development. Lower thermogenic capacity may also result from less mature muscle fibres of low incubation temperature ducklings as muscle maturity plays an important role in thermoregulation (Hohtola & Visser 1998). Indeed, a previous study demonstrated that low-temperature ducklings have reduced locomotor performance, a trait also affected by muscle maturity (Hohtola & Visser 1998), than ducklings incubated at higher temperatures determined by measuring velocity of ducklings while swimming and running (Hopkins *et al.* 2011).

Thermoregulation is essential to survival of precocial avian offspring; hypothermia accounts for 8–9% of mortality of mallards (*Anas platyrynchos*) and 24–25% of canvasbacks (*Aythya valisineria*) in the first days after hatching (Talent, Jarvis & Krapu 1983; Mauser, Jarvis & Gilmer 1994; Korschgen *et al.* 1996). High mortality because of hypothermia is probably the case with wood ducks as well, as up to 95% of early wood duck mortality occurs within 2 weeks of hatching (Bellrose & Holm 1994). In more

northerly populations, mortality is often greater in broods hatched early in the nesting season, presumably because these ducklings are exposed to more severe weather (Bellrose & Holm 1994). Because ducklings are frequently exposed to ambient temperatures below thermoneutral, ducklings that are less efficient at thermoregulating may have reduced capacity for other energetically costly processes, such as growth and immune function, as duckling metabolic rates were 155–250% greater during the thermal challenge than at thermoneutral. It is also important to note that we detected differences in respiration rates when ducklings were exposed to a relatively mild thermal challenge (15 °C); therefore, the magnitude of differences in respiration when thermoregulating should be exacerbated during more severe thermal challenge temperatures. Growing larger rapidly, thus reducing the SA/V ratio, is perhaps one of the best strategies for reducing the cost of homeothermy in precocial waterfowl. However, the ducklings expending more energy to thermoregulate may not be capable of offsetting the energetic demands of thermoregulating while concomitantly allocating more resources towards early growth.

Avian ecologists have long appreciated the role incubation temperature plays in hatching success and the length of incubation. Recently, however, theory on the evolution of avian clutch size has begun to incorporate incubation temperature as an important selective factor (Cooper *et al*. 2005; Martin 2008). Such evolutionary frameworks focus primarily on the relationships among incubation temperature, seasonal and latitudinal variations in clutch size, egg size and incubation period, and how these relationships subsequently influence hatching success. Our research suggests that perhaps there is an additional layer of complexity to the relationships among nest temperature, egg size and clutch size, as incubation temperature may also act as an important source of phenotypic variation among offspring within a population.

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