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Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings

S. E. DuRant[1], G. R. Hepp[2], I. T. Moore[3], B. C. Hopkins[1] and W. A. Hopkins[1,*]

[1]Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA, [2]School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, USA and [3]Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

*Author for correspondence (hopkinsw@vt.edu)

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SUMMARY

Early developmental experiences, such as incubation conditions, can have important consequences for post-hatching fitness in birds. Although the effects of incubation temperature on phenotype of avian hatchlings are poorly understood, recent research suggests that subtle changes in incubation conditions can influence hatchling characteristics, including body size and condition. We designed an experiment to explore the effects of incubation temperature on hatching success, survival to 9 days post hatch, growth and the hypothalamo–pituitary–adrenal (HPA) axis in wood ducks (*Aix sponsa*). Wood duck eggs were collected from nest boxes and experimentally incubated at three temperatures (35.0, 35.9 and 37.0°C), each falling within the range of temperatures of naturally incubated wood duck nests. Survival and growth were monitored in ducklings fed *ad libitum* for 9 days post hatch. In addition, baseline and stress-induced plasma corticosterone concentrations were measured in 2 and 9 day old ducklings. Hatching success and survival to 9 days was greatest in ducks incubated at the intermediate temperature. Ducklings incubated at 35.9°C and 37.0°C had 43% higher growth rates than ducklings incubated at 35.0°C. In addition, ducklings incubated at 35.0°C had higher baseline (17–50%) and stress-induced (32–84%) corticosterone concentrations than ducklings incubated at 35.9°C and 37.0°C at 2 and 9 days post hatch. We also found a significant negative correlation between body size and plasma corticosterone concentrations (baseline and stress-induced) in 9 day old ducklings. To our knowledge, this is the first study to demonstrate that thermal conditions experienced during embryonic development can influence the HPA axis of young birds. Our results illustrate that subtle changes (<1.0°C) in the incubation environment can have important consequences for physiological traits important to fitness.

Key words: maternal effects, corticosterone, incubation.

INTRODUCTION

Maternal effects are maternal traits that influence offspring phenotype *via* non-genetic pathways (Mousseau and Fox, 1998). In many cases maternal effects can have profound influences on offspring, sometimes rivaling the effects of genomic contributions to offspring fitness (Bernardo, 1996; Price, 1998). In fact, recent reviews have highlighted the importance of such early developmental experiences because of the influence they can have on subsequent life history decisions that ultimately influence survival and reproductive success (Lindstrom, 1999; Metcalfe and Monaghan, 2001). Maternal effects (e.g. mate choice, nest-site selection, incubation behavior, nutrient allocation to embryo, etc.) have been widely studied in a variety of organisms, including birds, and have been shown to affect a multitude of offspring characteristics that are important to their success. Such effects include time to hatching, size at hatching, hatchling growth rate, begging behavior, immunocompetence and secondary sexual characteristics (Schwabl, 1996; Price, 1998; Saino et al., 2005).

In birds, an important maternal effect is incubation behavior, a critical component of avian reproduction. Incubation influences both the current reproductive success and future fitness of the parent (Gloutney et al., 1996; Heaney and Monaghan, 1996; Williams, 1996; Bryan and Bryant, 1999; Reid et al., 2000; Visser and Lessels, 2001; Tinbergen and Williams, 2002). Egg incubation is a demanding period because parents are confronted with the conflicting demands of simultaneously maintaining their own body condition (White and Kinney, 1974; Carey, 1980; Deeming, 2002) and the environmental conditions of the nest (Webb, 1987; Deeming, 2002). The demands of incubation can be particularly severe in species that display uniparental incubation, particularly when the incubating parent receives no aid in incubating the eggs or procuring its own food. Time spent foraging away from the nest can decrease the temperature at which eggs are incubated; thus, slowing their development and subsequently increasing the duration of incubation (Lyon and Montgomerie, 1985; Deeming and Ferguson, 1991; Zicus et al., 1995; Neuchterlein and Buitron, 2002; Martin et al., 2007). Extended incubation duration can incur risks, including increased susceptibility to egg predation (Reid et al., 2002; Tombre and Erikstad, 1996).

In addition to influencing the probability of egg predation, changes in the incubation environment also can influence offspring phenotype. In reptiles, incubation temperature has been shown to influence traits important to population dynamics and individual fitness, including offspring sex ratios, body size, body morphology, growth rate, locomotor performance, behavior and survival (Bull, 1980; Joanen et al., 1987; Burger, 1990; Brooks et al., 1991; Van Damme et al., 1992; Shine et al., 1997; Angilletta, 2000; Booth et al., 2000; Nelson et al., 2004). By contrast, the effect of incubation conditions on hatchling fitness in birds is poorly understood. However, recent research suggests that subtle changes in incubation

conditions can have serious effects on hatchling characteristics. Hepp et al. (Hepp et al., 2006) investigated the effects of incubation temperature on the duration of embryonic development and offspring phenotype in wood ducks (*Aix sponsa*) by artificially incubating eggs at three temperatures (34.6, 36.0 and 37.6°C) within the range of temperatures found in naturally incubated nests [range 34.8–37.7°C (Manlove and Hepp, 2000; Folk, 2001; Hepp et al., 2006)]. They found that incubation at lower temperatures significantly extended embryonic development, with eggs incubated at 34.6°C hatching nearly 10 days later than eggs incubated at 37.6°C. Further, proximate analysis revealed that ducklings from eggs incubated at lower temperatures had significantly lower wet and dry mass, lower protein content and higher ash content than ducklings incubated at the higher temperatures (Hepp et al., 2006).

Recent research suggests that conditions experienced by avian embryos during incubation can also influence a hatchling's stress physiology. Two studies observed significant differences in stress-induced corticosterone (the primary stress hormone in birds) concentrations between groups of hatchlings that experienced different incubation conditions, suggesting that these effects were mediated through the behavior or physiology of the parents (Walker et al., 2005; Cyr et al., 2007) (see also Yahav et al., 2004). Walker et al. found that newly hatched Magellanic penguin (*Spheniscus magellanicus*) chicks from frequently disturbed (tourist visited) areas had similar baseline corticosterone concentrations as chicks in unvisited areas (Walker et al., 2005). However, chicks from disturbed areas expressed significantly greater stress-induced free corticosterone levels after capture compared with chicks from undisturbed areas. Although the authors attributed these effects to cues relayed to the offspring from the parent during incubation, the possibility exists that these effects were attributable to events that occurred prior to egg laying (e.g. steroid deposition to the yolk). A recent study on European starlings (*Sturnus vulgaris*) also provided evidence that incubation conditions can influence the hypothalamo–pituitary–adrenal (HPA) axis (Cyr et al., 2007). Adult starlings exposed to a stressor while they were incubating eggs produced chicks that, at 18 days post hatch, displayed higher levels of stress-induced free corticosterone than chicks produced by undisturbed parents. In that study, females were exposed to a chronic stress protocol (CSP; a rotation of four stressors per day, spaced 1–2 h apart) for 8 days while they were incubating eggs. Females left the nest when stressors were present and typically did not return until the stressor was removed (30 min later). Because the CSP was present only during incubation, these data suggest that the effects of the CSP on the chicks were attributable to either (1) alterations in the female's behavior or physiology during incubation (e.g. reduced time on nests, change in maternal body temperature or heart rate) or (2) from changes in parental care post-incubation.

In this study, we used wood ducks (*Aix sponsa*) to investigate whether variation in incubation temperature alone can affect important post-hatching characteristics of hatchlings. Specifically, we evaluated whether slight differences in incubation temperature, which is largely dependent on female behavior, would influence early survival, growth, body condition and the HPA axis of ducklings. We randomly allocated eggs from natural nests to one of three incubation temperatures (35.0, 35.9 and 37.0°C). We monitored survival and growth to 9 days post hatch and measured baseline and stress-induced corticosterone in ducklings at 2 and 9 days post hatch. Based on the aforementioned studies (Hepp et al., 2006; Walker et al., 2006; Cyr et al., 2007), we predicted that ducklings incubated at the lowest temperature would have lower survival and growth, and higher corticosterone concentrations than ducklings incubated at the higher temperatures.

MATERIALS AND METHODS

Study species

The wood duck (*Aix sponsa* L.) is a widely distributed dabbling duck whose breeding range extends throughout much of the eastern half of North America and along the west coast from southern California to British Columbia (Hepp and Bellrose, 1995). Wood ducks are relatively small-bodied (~650–700 g) and occupy a great diversity of aquatic habitats, including freshwater marshes, wooded swamps, beaver ponds and bottomland habitats along major tributaries. Female wood ducks nest in tree cavities but will also use artificial nest boxes, a factor that facilitates locating nests and capturing females (Hepp et al., 1987b). Although they are socially monogamous and begin to form pair bonds in autumn and winter, only the female incubates the eggs and cares for the young (Hepp and Bellrose, 1995). During this time she receives no aid from the male. Females lay one egg per day, and the mean clutch size is 12 eggs (Bellrose and Holm, 1994). Night incubation begins when ~75% of the clutch is laid, and 24 h incubation doesn't begin until egg laying is complete (Hepp and Bellrose, 1995). Minimum, maximum and mean temperatures of naturally incubated wood duck nests at our study site were 34.98, 38.70 and 36.79°C, respectively (G.R.H., unpublished data). In our study area, wood ducks initiate nesting in mid-late February and continue nesting until mid-July. Females can produce multiple broods in a breeding season.

Study site

Eggs were collected from aquatic habitats located on the Department of Energy's Savannah River Site (SRS) in west-central South Carolina. Nest boxes are located throughout the SRS on two large reservoirs (N=120) and 10 isolated wetlands (N=81). These nest boxes have been available to wood ducks for >10 years and have been monitored every year by staff and graduate students from the Savannah River Ecology Laboratory. Each year >100 nests are initiated in these nest boxes. There are at least 100 breeding females using the sites and >2500 eggs are laid annually (R. Kennamer, personal communication).

Egg collection and incubation

To collect fresh, non-incubated eggs, nest boxes were checked every 4 days during the breeding season until nests were initiated. Active nests were visited daily, and new eggs were collected, individually marked and stored at 20°C and 55–60% humidity until they were placed in incubators. Avian embryos do not develop when maintained below 24–27°C (White and Kinney, 1974) and holding duck eggs for <5 days at low temperatures before incubation does not affect hatchability (Arnold et al., 1987). Wooden eggs were used to replace wood duck eggs so that females would continue to lay eggs and would not abandon nests (Hepp et al., 1987a).

After 4 days of collection, eggs were transported to Virginia Tech and artificially incubated in Grumbach incubators (model BSS 160, Asslar, Germany) at one of three temperatures (35.0, 35.9 and 37.0°C), which produced three incubation durations (37±0.19, 35±0.15 and 31±0.27 days, respectively). Humidity was maintained at 60–65% in each of the three incubators. Although experimental mean temperatures were achieved, incubators were not maintained at constant temperatures. Instead, incubators were programmed to allow two cool-down periods each day (~3°C reduction in mean temperature for 75 min at 08:15 h and 18:30 h) to simulate natural daily feeding recesses taken by mothers during incubation (Manlove

and Hepp, 2000). Such periodic chilling of the eggs generally results in increased hatchability (Landauer, 1967). Throughout incubation we candled eggs every 7–10 days to check for embryonic mortality. Stage at death was determined using age criteria of excised wood duck embryos from Bellrose and Holm (Bellrose and Holm, 1994).

Duckling husbandry

After hatching, all ducklings were housed under identical conditions. Ducklings were maintained communally in 46 × 32 × 24.5 cm plastic cages (2–3 ducklings per cage) arranged in a rack system in a temperature-controlled environmental chamber held at 28°C. Ducklings were allowed constant access to food (ground-up Dumor Chick Starter/Grower 20%, Tractor Supply Co., Brentwood, TN, USA) and water. Ducklings were warmed by a 50 W infrared light bulb suspended 32.5 cm above the bottom of the cage (creating a thermal gradient) and held on a 14 h:10 h L:D photoperiod. Each morning all cages, food and water dishes were cleaned. At the end of the experiment, ducklings were killed *via* asphyxiation with carbon dioxide followed by cervical dislocation in accordance with IACUC standards.

Duckling growth and survival

We monitored duckling growth by weighing ducklings every morning during cage cleaning. We calculated daily growth rate of ducklings by subtracting their hatch mass (day 0) from their mass at 9 days post hatch and dividing by nine (the total number of days elapsed). Tarsus length was measured as the distance between the notch at the intertarsal joint and the juncture of the tarsometatarsus and the third digit. Tarsus length was measured at hatching and on days 2 and 9 post hatch. We checked for duckling mortality each morning and evening.

Stress hormone protocol

To determine whether incubation temperature influences the HPA axis, baseline and stress-induced corticosterone concentrations were measured in 2 and 9 day old ducklings from each of the three incubation temperature treatments (35.0, 35.9 and 37.0°C). We used a repeated-measures design; therefore, all blood samples were taken from the same individuals at both ages. Ducklings were bled *via* the femoral vein immediately after removal from their cage (<3 min) then held in a cloth bag. Ducklings were bled again at 15 min post removal from their cage. To control for circadian influences on plasma corticosterone levels, all samples were collected between 12:50 h and 15:45 h. Blood was collected in heparinized capillary tubes and stored on ice for no longer than 1.5 h before separating plasma *via* centrifugation at 3.5 ***g***. Plasma samples were then stored at –80°C.

Radioimmunoassay

Plasma corticosterone was assayed by radioimmunoassay (RIA) techniques following extraction based on the methods of Wingfield et al. (Wingfield et al., 1992). To determine sample extraction efficiency we equilibrated each sample overnight with 2000 c.p.m. of tritiated steroid. Each sample was extracted with 4 ml of dichloromethane, dried using nitrogen gas and resuspended in 600 µl of phosphate buffered saline. Individual extraction efficiency was determined from 100 µl of the sample (mean recoveries were 86.5% and 82.7% for assay I and assay II, respectively). Two hundred µl of the sample was allocated to each of two duplicates for the assay. Then, duplicate samples were compared with a standard curve, which contained known amounts of corticosterone, run with each assay. Inter- and intra-assay variation were 17 and 9.6%, respectively, as determined by running standards in each assay.

Statistical analyses

All statistical analyses were run in SAS 9.1 (SAS Institute, Cary, NC, USA) and statistical significance was recognized at $\alpha<0.05$. In the few instances where multiple individuals from a single clutch were in the same treatment group, we randomly selected one of the individuals to be used in analyses to avoid pseudo-replication. Where appropriate, we tested for normal distribution of the data and homoscedasticity using Ryan–Joiners and Bartlett's tests, respectively.

Hatching success and post-hatch survival were binary data and therefore were analyzed using a Chi Squared test and Fisher's exact test (SAS proc freq), respectively. Because the hatching success and post-hatch survival data set included individuals from the same clutch, we initially included clutch as a random effect. Clutch had no significant effect on hatching success of fertilized eggs or post-hatch survival and was eventually dropped from the model because models excluding this variable were a better fit to the data set based on AIC values. We determined the effects of incubation temperature on the duration of incubation using a one-way analysis of variance (ANOVA; SAS proc glm).

To ensure that egg mass did not differ among treatments we conducted a one-way ANOVA (SAS proc glm). In addition, we tested for differences in duckling mass at hatching among treatments using a one-way analysis of covariance (ANCOVA) with egg mass as the covariate. Differences in growth rates among incubation temperature treatments were assessed using a one-way ANOVA (SAS proc glm). Only individuals that survived to 9 days post hatch were included in the analysis, and all growth data met assumptions of normality and homoscedasticity. Growth trajectories were compared among treatments using a repeated-measures ANOVA (SAS proc glm). The model included incubation temperature, age and their interaction as main effects and only included individuals that survived to 9 days post hatch. Not all ducklings were weighed on day 1 and day 2; therefore, these days were omitted from the model.

To estimate body condition we plotted body mass against tarsus length. Differences between treatments in body condition of 9 day old ducklings were evaluated using an ANCOVA (SAS proc glm) with incubation temperature as the independent variable, mass as the dependent variable and tarsus length as the covariate.

Effects of incubation temperature on the HPA axis were examined using a mixed-model ANOVA (SAS proc mixed), including only individuals for which we had baseline and stress-induced data points at both ages. All data met assumptions of normality but plasma corticosterone concentrations were $\log_{10}$-transformed to better fit assumptions of homoscedasticity. Incubation temperature, age, stress (baseline *vs* stress-induced), and all interactions between these variables were included as main effects in the initial model. Non-significant interactions were dropped from subsequent iterations of the model. Because plasma samples were collected from the same individuals at both time points (baseline and stress-induced) and ages, individual was included in the statistical model as a random effect to account for non-independence of plasma corticosterone concentrations. Differences in the magnitude of the stress response among treatment groups were also evaluated by comparing the factorial increase in plasma corticosterone concentration across treatments and age groups using a repeated-measures ANOVA (SAS proc glm). Finally, we used Pearson correlation coefficients to examine relationships between body mass and body condition *vs* $\log_{10}$-transformed plasma corticosterone concentrations, both baseline and stress-induced. In these analyses, the residuals of a regression of mass on tarsus length were used as our measure of body condition.

Table 1. Means of incubation duration, hatching success, survival to 9 days post hatch, egg mass, hatch mass and growth rates (g day^{-1}) of wood ducks incubated at one of three temperatures (35.0, 35.9, 37.0°C)

	Incubation temperature						
	35.0°C		35.9°C		37.0°C		
Variable	Means (±s.e.m.)	*N*	Means (±s.e.m.)	*N*	Mean (±s.e.m.)	*N*	*P*-value
Incubation duration	31 (0.27)	52	35 (0.15)	55	37 (0.19)	28	<0.001
% Hatching	43	52	67	55	50	28	0.073
% Survival to 9 days post hatch	70	23	92	36	76	14	0.074
Egg mass	41.3 (0.71)	23	40.3 (0.62)	36	38.9 (0.60)	14	0.109
Hatch mass*	24.9 (0.07)	23	24.4 (0.06)	36	24.8 (0.11)	14	0.943
Growth rate (g day^{-1})	2.3 (0.29)	15	3.3 (0.22)	27	3.3 (0.26)	12	0.009

*LS means are reported for duckling mass at hatching because egg mass was included as a covariate in the statistical model.

RESULTS

There was a trend towards higher hatching success of eggs incubated at the medium temperature (P=0.073; Table 1). Eggs incubated at this temperature had 51% and 34% higher hatching success than the eggs incubated at the low and high temperatures, respectively. A similar trend was seen when examining the effects of incubation temperature on post-hatching survival to 9 days (P=0.074; Table 1). Post-hatching survival for ducklings from the medium incubation temperature was 32% and 22% greater than for ducklings that hatched from eggs incubated at the low and high temperatures, respectively. All of the duckling mortality in the high and intermediate temperature groups occurred within 3 days of hatching whereas 71% of ducklings that hatched from eggs incubated at the lowest temperature died >4 days post hatch. Incubation temperature significantly affected the duration of incubation ($F_{2,72}$=171.56; P<0.001; Table 1), with higher temperatures inducing more rapid development. *Post hoc* comparisons (Tukey HSD) revealed that all the treatments differed significantly from one another in duration of incubation.

There were no differences in egg mass among treatments (P=0.109; Table 1). Egg mass correlated strongly with duckling mass at hatching ($F_{1,67}$=159.30; P<0.001) but duckling mass at hatching did not differ among treatment groups (P=0.943; Table 1). Ducklings from all incubation temperatures had positive growth rates and gained 23–33 g from hatching to 9 days post hatch. However, ducklings from the medium and high incubation temperatures both had 43% higher growth rates than ducklings from the low treatment group ($F_{2,47}$=5.23; P=0.009; Table 1). Changes in body mass were not apparent until 5 days post hatch (incubation temperature × age: $F_{14,47}$=3.90; P=0.008; Fig. 1). By 9 days post-hatch, ducklings that hatched from eggs incubated at the high and intermediate temperatures were 17% larger than ducklings that hatched from eggs incubated at the lowest temperature.

Tarsus length correlated strongly with body mass ($F_{1,47}$=66.21; P<0.001), and there was a significant effect of incubation temperature on body condition at 9 days post hatch ($F_{2,47}$=4.25; P=0.020; Fig. 2). There was no temperature by tarsus interaction (P=0.728). *Post hoc* comparisons (Tukey HSD) revealed that ducklings that hatched from eggs incubated at the medium and high temperature treatments were heavier in relation to tarsus length (i.e. had higher body condition) than ducklings that hatched from eggs incubated at the lowest temperature. For visual purposes these data are presented as mean (±s.e.m.) residuals of mass on tarsus (Fig. 2).

Capture and restraint resulted in a 140–262% increase in plasma corticosterone in 2 and 9 day old ducklings from all incubation temperatures (stress: $F_{1,140}$=105.13; P<0.0001; Fig. 3). There also was a significant effect of age on baseline and stress-induced plasma corticosterone concentrations (age: $F_{1,140}$=24.99; P<0.0001). Specifically, both baseline and stress-induced plasma corticosterone concentrations were lower at 9 days post hatch than at 2 days post hatch. Moreover, incubation temperature had a significant effect on the HPA axis of both 2 and 9 day old ducklings ($F_{2,140}$=4.98; P=0.008). Ducklings from the lowest incubation temperature treatment had higher baseline (17–50%) and stress-induced (32–84%) corticosterone concentrations than ducklings that hatched from eggs incubated at the intermediate and high temperatures at both 2 and 9 days post hatch. Ducklings from the intermediate incubation temperature had the lowest plasma corticosterone concentrations (baseline and stress-induced) at day 2. However, at 9 days post-hatching, ducklings from the high incubation temperature had similar corticosterone profiles as ducklings from the intermediate temperature whereas ducklings from the low incubation temperature continued to exhibit higher levels of both baseline and stress-induced plasma corticosterone concentrations. There was no effect of age, incubation temperature or their interaction on the factorial increase in plasma corticosterone concentrations (P>0.179 in all cases).

We also found a significant negative correlation between body mass and plasma corticosterone concentrations (baseline: R=–0.284, P=0.048; stress-induced: R=–0.294, P=0.040) in 9 day old ducklings (Fig. 4A,B). A similar trend existed between tarsus length and plasma

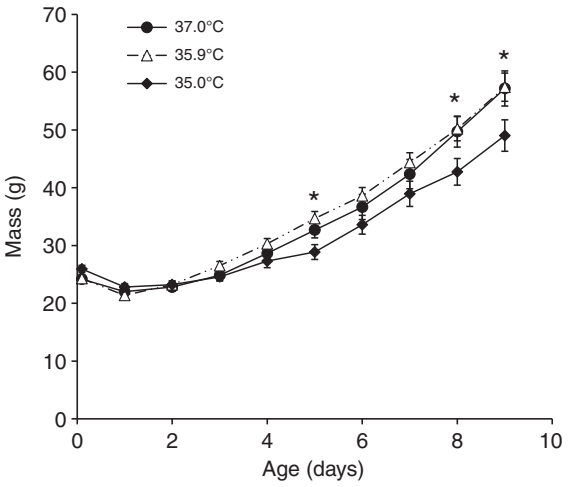

Fig. 1. Patterns of growth among wood duck (*Aix sponsa*) ducklings that hatched from eggs incubated at one of three temperatures (35.0, 35.9 or 37.0°C). Only ducklings that survived to 9 days post hatch are included. Error bars are ± 1 standard error of the mean. 35.0°C: N=12; 35.9°C: N=27; 37.0°C: N=15. *Denotes when significant treatment effects were detected in *post hoc* analyses of the overall statistical model.

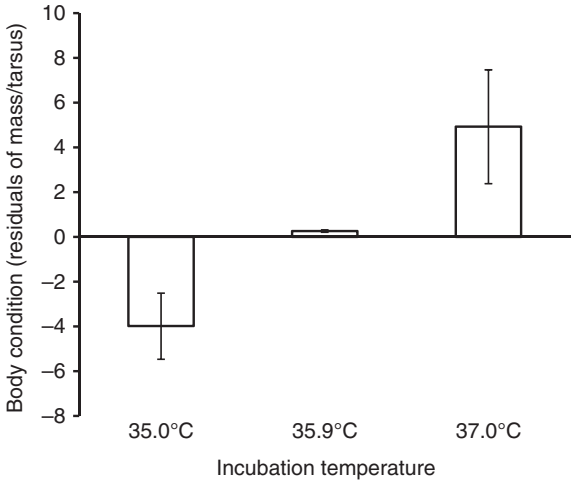

Fig. 2. Body condition (residuals of body mass/tarsus length) of 9 days post hatch wood duck (*Aix sponsa*) ducklings that hatched from eggs incubated at one of three temperatures (35.0, 35.9 or 37.0°C). Error bars are ± 1 standard error of the mean. 35.0°C: *N*=15; 35.9°C: *N*=26; 37.0°C: *N*=10. Differences in body size among treatments were examined using ANCOVA with mass as the independent variable and tarsus as the covariate.

corticosterone (baseline: R=–0.308, P=0.032; stress-induced: R=–0.248, P=0.085) in ducklings that were 9 days post hatch (data not shown). There was no significant relationship between body mass or tarsus length and plasma corticosterone concentrations in ducklings that were 2 days post hatch (in all cases P>0.37; data not shown) and there was no significant relationship between body condition and plasma corticosterone concentration in either age group (in all cases P>0.20; data not shown).

DISCUSSION

We found that small differences in incubation temperature, within the temperature range of naturally incubated nests, can affect a suite

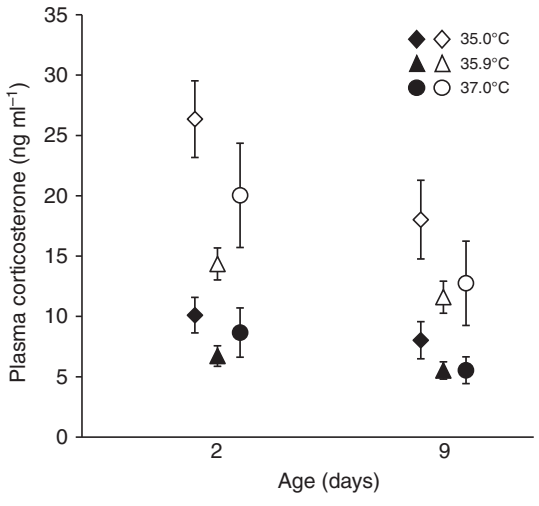

Fig. 3. Plasma corticosterone concentrations (ng ml^{-1}) of wood duck (*Aix sponsa*) ducklings that hatched from eggs incubated at one of three temperatures (35.0, 35.9 or 37.0°C). Closed symbols represent baseline plasma corticosterone concentrations and open symbols represent stress-induced corticosterone concentrations. The same individuals were bled at each data point. Error bars are ± 1 standard error of the mean. 35.0°C: *N*=14; 35.9°C: *N*=25; 37.0°C: *N*=10.

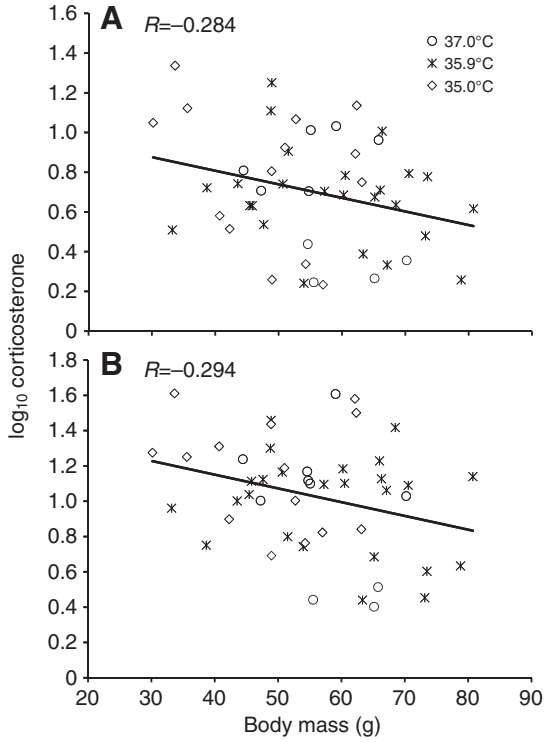

Fig. 4. Relationships between corticosterone concentrations (A: baseline; B: stress-induced) *versus* body mass in 9 days post hatch wood duck (*Aix sponsa*) ducklings that hatched from eggs incubated at one of three temperatures (35.0, 35.9 or 37.0°C). 35.0°C: *N*=14; 35.9°C: *N*=25; 37.0°C: *N*=10.

of important post-hatching characteristics in wood ducks. Ducklings that hatched from eggs incubated at the lowest temperature had lower survival, slower growth, lower body condition and higher baseline and stress-induced corticosterone than ducklings incubated at the higher temperatures. To our knowledge, this is the first study in a wild bird to demonstrate that slight variations in the incubation environment can affect the growth, development and physiology of the hatchlings.

Although ducklings from all treatment groups were similar in size at hatching, by 9 days after hatching ducklings from the lowest incubation temperature were 15% smaller and were in poorer body condition than ducklings incubated at higher temperatures. The lack of treatment effect on hatchling mass in our study was surprising as there was a clear effect of incubation temperature on hatchling mass in Hepp et al. (Hepp et al., 2006). Body size is important for survival in young birds, as smaller body size can decrease their ability to recover from mass loss and their ability to compete with conspecifics during rapid growth phases (Arroyo, 2002), and could increase their susceptibility to gape-limited predators (e.g. fish). Additionally, ducklings of larger body size have reduced mass-specific energetic demands when maintaining homeothermy. In many animals, juvenile body size also is an important contributor to future survival and reproduction (Semlitsch et al., 1988; Ringsby et al., 1998; Van der Jeugd and Larsson, 1998; Monros et al., 2002; Altwegg and Reyer, 2003). For example, larger offspring are often more likely to survive strenuous events [e.g. over-wintering, migration (Milner et al., 1999; Bodie and Semlitsch, 2000; Munch et al., 2003)] and reach maturity more quickly than offspring of smaller body size (Dawson and Clark, 2000). Therefore, it is possible that eggs incubated at lower temperatures produced offspring that are less likely to survive their

first year or may begin breeding later than ducklings that hatched from eggs incubated at the higher temperatures. However, future studies are needed to evaluate these hypotheses.

We also found that incubation temperature strongly influenced the HPA-axis in ducklings at both 2 and 9 days post-hatching. At 2 days post-hatching, ducklings incubated at the lowest temperature had higher baseline and stress-induced corticosterone concentrations than ducklings incubated at the medium and high temperature, respectively. Although all of the ducklings exhibited a decrease in baseline plasma corticosterone concentrations from day 2 to day 9, ducklings from the lowest temperature continued to express both higher baseline and stress-induced plasma corticosterone concentrations than ducklings incubated at the higher temperatures. The ontogenetic shift in plasma corticosterone concentrations we saw in wood duck ducklings is consistent with patterns of plasma corticosterone in Mallard (*Anas platyrhynchos*) ducklings; Mallards had higher baseline and stress-induced corticosterone 1 day after hatching than at 7 days after hatching (Holmes et al., 1989). Interestingly, wood duck ducklings in this study did not appear to undergo a refractory period, a brief period of unresponsiveness to stressors that occurs shortly after hatching/birth, thought to protect young from the detrimental effects of elevated glucocorticoids (reviewed in Sapolsky and Meaney, 1986). However, it is plausible that a refractory period may have occurred prior to our first measurements on day 2.

Our finding that incubation temperature influences the HPA axis of hatchling ducks has many implications for their future development. Corticosterone is an extremely important hormone because it affects a multitude of physiological systems in vertebrates [e.g. reproductive physiology, immune function, cardiovascular function, metabolism (Shreck, 1993; Dhabar and McEwen, 1999; Moore and Jessop, 2002; van den Buuse et al., 2002)] and depending on the context, it may have stimulatory, inhibitory or permissive effects (Sapolsky et al., 2000). There is evidence among vertebrates that high levels of baseline corticosterone early in ontogeny can have negative effects on growth (Morici et al., 1997; Spencer et al., 2003), immune function (Morici et al., 1997), neural development (Caldjii et al., 2001) and cognitive function (Kitaysky et al., 2003; Kitaysky et al., 2006). Although the underlying cause of differences in corticosterone concentrations among treatments in our study remains unclear, one possibility is that ducklings incubated at the lowest temperature are developmentally delayed compared with ducklings from eggs incubated at the higher temperatures. If this is the case, then we would expect ducklings from the lowest incubation temperature to eventually express plasma corticosterone profiles that are similar to ducklings incubated at the higher temperatures. However, longer-term studies that monitor baseline and stress-induced corticosterone concentrations throughout adolescence would be needed to directly address this hypothesis. Ideally, such studies could also be conducted on free-living birds to determine how these physiological responses manifest under natural conditions.

An interesting finding was that baseline and stress-induced plasma corticosterone concentrations at 9 days of age were negatively correlated with body mass and tarsus length. Many studies have found negative correlations between aspects of body size (e.g. body mass, body condition) and baseline and/or stress-induced corticosterone (Schoech et al., 1997; Kitaysky et al., 1999; Moore et al., 2000; Romero and Wikelski, 2001; Perfito et al., 2002). Our failure to detect a significant correlation between morphology and corticosterone concentrations in ducklings at 2 days post-hatching may reflect the small range of body sizes at this age. Although we cannot determine whether corticosterone is directly influencing body size or *vice versa*, previous studies have shown that exposure of young animals to exogenous corticosterone can inhibit growth (Beckett et al., 1996; Morici et al., 1997; Spencer et al., 2003; Kilic et al., 2008; Wada and Breuner, 2008). Such findings suggest that high baseline corticosterone concentrations of ducklings incubated at the low temperature may have contributed to the slower growth and reduced body sizes we observed in this treatment group. Again, however, 9 day old ducklings incubated at the lowest temperature may be developmentally younger than ducklings incubated at higher temperatures. Retarded development might account for both their smaller body size and higher plasma corticosterone concentrations compared with ducklings incubated at the higher temperatures. This may also explain why we did not detect a relationship between corticosterone and body condition. Body size and corticosterone may be tightly linked with developmental stage whereas body condition may not.

Biologists have long appreciated the importance of maternal condition during reproduction and the adaptive interplay between maternal and offspring health (Trivers and Willard, 1973). Indeed, maternal effects are known to have profound effects on offspring, often rivaling the effects of genomic contributions to offspring fitness (Bernardo, 1996; Price, 1998). Several recent reviews have highlighted the importance of such early developmental experiences because of the effects they can have on subsequent life-history decisions that ultimately affect survival and reproductive success (Lindstrom, 1999; Metcalfe and Monaghan, 2001). Only recently, however, have we begun to appreciate the role that female incubation behavior in birds might play in shaping the phenotype of her offspring (Hepp et al., 2006; Walker et al., 2005; Cyr et al., 2007). Female behavior plays a large role in determining the environment of the nest, because her attendance positively affects nest temperatures (Martin et al., 2007). Our study clearly demonstrates that even small changes in nest temperature caused by variation in incubation behavior can have dramatic effects on the physiology of hatchlings. If these sub-lethal effects translate into differences in survival or recruitment then there may be strong selection on females to maintain a narrow range of nest temperatures. Furthermore, sub-optimal foraging conditions or frequent disturbance may reduce female reproductive success by forcing females to allocate time away from incubation.

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