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Maternal effects in Wood Ducks: incubation temperature influences incubation period and neonate phenotype

G. R. HEPP,*† R. A. KENNAMER‡ and M. H. JOHNSON§

**School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, ‡Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29801, and §Department of Biostatistics AE-3035, Medical College of Georgia, Augusta, GA 30912-4900, USA*

Summary

1. Maternal effects often explain a significant amount of variation in offspring phenotype, and can be important in the evolution of life histories. Incubation of eggs is an important maternal effect, and optimal growth and development of avian embryos takes place within a narrow range of incubation temperatures, but the effect of incubation microclimate on neonate phenotype remains relatively unexplored in birds.

2. In this study of Wood Ducks (*Aix sponsa* Linnaeus) we examined effects of incubation temperature on the length of incubation period and neonate quality. We monitored nest temperatures and incubation periods of naturally incubated Wood Duck nests and found a strong inverse relationship between incubation period and average nest temperature.

3. Next, we collected three unincubated eggs from each of 48 nests, and randomly assigned eggs from each nest to one of three incubation temperatures (34·6, 36·0 and 37·4 °C). Experimental incubation temperatures overlapped average nest temperatures of naturally incubated Wood Duck nests.

4. Hatching success varied with incubation temperature and was lowest for eggs incubated at the lowest temperature. Incubation period of experimental eggs decreased with increasing temperature but was not affected by fresh egg mass.

5. Wood Duck embryos catabolized an estimated 34–38% of egg lipids and 25–33% of egg protein during incubation. Percentage change of lipids increased with decreasing incubation temperature, but not significantly. Embryos incubated at lower temperatures used a greater percentage of protein than embryos incubated at higher temperatures.

6. In analyses using fresh egg mass as the covariate, we found that wet and dry mass of ducklings increased with increasing incubation temperature. Decreases in lipid content of Wood Duck neonates with decreasing incubation temperature were not significant, but eggs incubated at low temperatures produced ducklings that had reduced protein mass and that were structurally larger than ducklings from eggs incubated at high temperatures.

7. Our study illustrates the importance of incubation temperature on the development of Wood Duck embryos. Decisions made by incubating parents that influence egg temperature can modify incubation period and offspring phenotype. Investigations of incubation as a reproductive cost should consider how parental decisions influence both parents and offspring.

Key-words: *Aix sponsa*, Anatidae, parental care, reproductive success

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Introduction

Neonates of most vertebrate populations vary phenotypically, and maternal effects often explain a significant amount of this phenotypic variation (Mousseau & Fox 1998). Maternal effects in birds may include nest-site selection, mate choice, egg size, incubation behaviour and brood care (Price 1998). Female birds, for example, control the amount of nutrients allocated to eggs, and egg size is often positively related to offspring size, growth rate and survival (Bolton 1991; Williams 1994; Pelayo & Clark 2003). Badyaev *et al.* (2002) reported the adaptive adjustment of offspring sex and growth

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†Author to whom correspondence should be addressed.
E-mail: heppgar@auburn.edu

characteristics by female House Finches (*Carpodacus mexicanus*); however, maternal effects are not always adaptive (Bernardo 1996).

Incubation is an important component of reproduction in birds. Most species have adopted contact incubation to maintain an environment appropriate for egg development (Deeming 2002a). However, incubating birds must balance the demands of self-maintenance with the care of developing eggs (White & Kinney 1974; Carey 1980; Deeming 2002b). In species with female-only incubation, such as waterfowl (Anatidae), the amount of time incubating females spend on the nest is influenced largely by body size (Afton & Paulus 1992; Deeming 2002c). Large-bodied species, generally with large nutrient reserves, are able to maintain greater incubation constancy than small-bodied species because they are less dependent on exogenous nutrients. A similar relationship within species also has been reported (Aldrich & Raveling 1983).

The environment needed for egg development deviates from optimal the longer parents are away from the nest. Reduced constancy can slow embryo development and lead to longer incubation periods (Deeming & Ferguson 1991; Zicus, Hennes & Riggs 1995). Increased incubation periods result in greater exposure of nests to predators, and may influence the phenotype of neonates. The effect of incubation microclimate on hatchling phenotypes has been studied widely in reptiles (Janzen 1993; Burger 1998; Booth, Thompson & Herring 2000), but remains relatively unexplored in birds (but see Booth 1987). Female Keelbacks (*Tropidonophis mairii*, Colubridae), for example, preferred laying eggs in wetter nest sites, and eggs incubated at wetter sites produced larger hatchlings that survived better than those incubated at drier sites (Brown & Shine 2004). Avian studies are needed that examine effects of incubation environment on neonate phenotype because of potential links between conditions experienced during early development and offspring fitness (Lindström 1999; Metcalfe & Monaghan 2001).

The Wood Duck (*Aix sponsa* Linnaeus) is a small-bodied, cavity-nesting species that relies heavily on exogenous sources of energy during incubation (Drobney 1980; Afton & Paulus 1992). Very little (7%) of the energy needed by incubating Wood Ducks is derived from catabolism of nutrient reserves (Afton & Paulus 1992). Females spend >80% of the day on the nest once full incubation begins, and take an average of two recesses per day (Manlove & Hepp 2000; Folk & Hepp 2003).

In this study we measured temperatures of naturally incubated Wood Duck nests and tested the relationship between average nest temperature and incubation period. Next, we selected temperatures approximating the range of average nest temperatures in naturally incubated nests, and experimentally tested effects of incubation temperature on incubation period, body mass and size, and body composition of newly hatched ducklings. Our results demonstrate the importance of incubation temperature on both incubation period and neonate quality.

Materials and methods

NEST TEMPERATURE AND INCUBATION PERIOD OF NATURALLY INCUBATED NESTS

We measured temperatures of Wood Duck nests at Eufaula National Wildlife Refuge (ENWR; 32° N, 85° W), located in south-west Georgia and south-east Alabama, USA. Nest boxes at ENWR were checked to monitor nesting activity in the breeding seasons of 2001 and 2002. Eggs were counted and numbered at each weekly visit. Temperature data loggers (Stowaway, Onset Computer Corp., Bourne, MA, USA) were installed in nests during early stages of egg-laying (median = two eggs) using the protocol of Manlove & Hepp (1998, 2000). Briefly, a platform containing a single wooden egg was placed in the nest box. A thermistor was embedded in each wooden egg, and the tip of the thermistor was exposed on top of the egg. Wooden eggs were fastened securely with lag bolts to each platform to prevent females from moving them, and were positioned in the centre of the clutch. A cable (61 cm) connected the thermistor to the data logger. Data loggers were placed beneath the nest, and recorded nest temperature every 6·4 min for 35 days. They were replaced if nests were monitored for >35 days. Data were downloaded and temperature data for each 24-h period were plotted. Examining data from graphs and spreadsheets provided an accurate method of determining when females arrived at and departed from nests during incubation (Manlove & Hepp 2000). We designated the first day of incubation as the day when females first spent portions of both day and night on the nest. Incubation period was the number of days from the onset of incubation to the day ducklings hatched. Data loggers revealed the day ducklings exited the nest box, and hatch date was assumed to be the previous day (Bellrose & Holm 1994). Average nest temperature was computed from the first day of incubation to hatch date.

We also used data from a long-term study of Wood Duck breeding ecology at the Department of Energy's Savannah River Site (SRS) in west-central South Carolina (33·1° N, 81·3° W) to estimate the incubation period of naturally incubated clutches ($n = 313$; Kennamer & Hepp 2000). During 1987–96 and 2003–04, eggs were candled within 8 days ($\bar{x} = 4·4 \pm 0·1$ SE) after the end of egg-laying (Kennamer, Harvey & Hepp 1990; Weller 1956). The stage of embryonic development was assessed to the nearest day using Hanson's (1954) guide for ageing incubated Wood Duck eggs. We estimated the day that incubation began by back-dating from these field-candling occasions. Hatching date was known precisely for nests in 1987 ($n = 76$) because we marked newly hatched ducklings as part of another study (Hepp, Kennamer & Harvey 1989). In 1988–96 and 2003–04, we estimated hatch dates only for nests that were visited on days when eggs were pipping or when ducklings had hatched and were in the nest ($n = 237$). Again, incubation period was the number of days from the start of incubation to the day ducklings hatched.

COLLECTION AND CARE OF EGGS

We collected Wood Duck eggs from nest boxes located on the SRS in west-central South Carolina (33·1° N, 81·3° W). Wood Ducks lay one egg per day, and clutch size averages 12 eggs (Drobney 1980; Bellrose & Holm 1994). We checked nest boxes weekly in March–April 2003 and 2004 to monitor nesting activity. We found nests during egg-laying, and used nests that contained one to four eggs when first discovered. All eggs were numbered, and nests were visited again for the next 2 or 3 days to count and number new eggs. Eggs were collected on the last visit to the nest. We collected three fresh, unincubated eggs from each nest. We limited collections to the early part of egg-laying because female Wood Ducks begin incubating eggs at night during the later part of egg-laying (≥7 eggs; Hepp 2004). Wood Ducks frequently engage in brood parasitism where more than one female deposits eggs in a nest (Clawson, Hartman & Fredrickson 1979; Semel & Sherman 1986). If more than one egg was deposited in a nest during 24 h, we randomly selected one of these eggs for use in the study.

Eggs were transported to Auburn University and placed in Roll-X-2 incubators (Lyon Electric Company, Los Angeles, CA, USA) within 24 h of being collected. Length, breadth (nearest 0·01 mm) and fresh mass (nearest 0·01 g) of eggs were measured, and eggs from each nest were assigned randomly to one of three incubation temperatures (34·6, 36·0 and 37·6 °C). Temperatures approximated the range of average nest temperatures that we recorded in naturally incubated Wood Duck nests. Location of eggs within incubators also was determined randomly. Incubator temperatures were measured every 15 min with Stowaway data loggers. Incubator temperatures also were monitored daily with a digital thermometer (Model TM99A, REOTEMP Instrument Corp., San Diego, CA, USA) and adjusted when necessary. We regularly monitored and maintained relative humidity (Thermo Recorder, Model TR-72S, T&D Corporation, Nagano, Japan) at 55–60% across temperature treatments.

Eggs were candled after 7–10 days to check viability (Weller 1956). Eggs failing to develop were removed from the incubator. As hatching date approached, eggs were candled every 12 h and hatching progress was recorded. Eggs that died during development were opened to determine the age of embryos (Bellrose & Holm 1994). We designated hatch date as the day when eggs reached the star-pipped stage. Star pipping takes place when the egg tooth first breaks through the shell, and is the earliest sign of hatching. We used the star-pipped stage to help standardize the hatch date for ducklings. Star-pipped eggs were removed from the incubator and weighed. Ducklings were extracted from eggs and dried with a paper towel. The wet weight (nearest 0·01 g), culmen and tarsus (nearest 0·01 mm with digital calipers) of ducklings were measured. Newly hatched ducklings were euthanized using CO_2 inhalation followed by cervical dislocation. All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2002-0361). Eggs were collected with permits issued by US Fish and Wildlife Service (MB748024-0) and South Carolina Department of Natural Resources (SREL-B10).

ESTIMATING COMPOSITION OF FRESH EGGS

Components of Wood Duck eggs vary isometrically with fresh egg mass (Hepp *et al.* 1987; Kennamer, Alsum & Colwell 1997). We used data and equations from Hepp *et al.* (1987) to estimate lipid, protein and water contents (g) of experimental eggs using log–log regressions of egg components (y) on fresh egg mass (x), $y = ax^b$. Egg protein was the sum of dry albumen and lean dry yolk, and egg water was the difference between fresh egg mass and the sum of the dry components (lipid = $0{\cdot}813x^{1{\cdot}12}$, 95% CI of $b = 0{\cdot}98$–$1{\cdot}26$, $r^2 = 0{\cdot}71$, $P < 0{\cdot}0001$; protein = $0{\cdot}184x^{0{\cdot}940}$, 95% CI of $b = 0{\cdot}86$–$1{\cdot}02$, $r^2 = 0{\cdot}83$, $P < 0{\cdot}0001$; water = $0{\cdot}213x^{1{\cdot}01}$, 95% CI of $b = 0{\cdot}97$–$1{\cdot}05$, $r^2 = 0{\cdot}96$, $P < 0{\cdot}0001$).

ANALYSIS OF DUCKLING BODY COMPOSITION

Wet ducklings were dried (60–65 °C) to constant mass, placed in plastic bags and frozen for ≈30 days. Water content was the difference between wet and dry mass of ducklings. Dry ducklings were ground, and neutral lipids were extracted from whole ducklings in a Soxhlet apparatus using petroleum ether (Dobush, Ankney & Krementz 1985). Lean samples were dried to constant mass, and lipid content was the difference in mass of the dry sample before and after extraction. Total lipid mass was calculated by multiplying the proportion of lipid in the dry, ground sample by dry mass of the duckling. Lean dry mass (LDM) was calculated by multiplying the proportion of non-lipid material in the dry, ground sample after lipid extraction by dry duckling mass. LDM samples were ground in a Wiley mill, redried, and duplicate samples were burned in a muffle furnace at 550 °C for 10 h to determine ash content. Differences in percentage ash between duplicate samples averaged 0·46 ± 0·04%, and means of duplicate samples were used in the analysis. Ash-free lean dry mass (AFLDM) was the difference between ash mass and LDM; total protein mass was calculated by multiplying the proportion of AFLDM in LDM by dry mass of the duckling. Total ash mass was the difference between dry duckling mass and the sum of total protein mass and total lipid mass.

STATISTICAL ANALYSIS

Regression analysis was used to test the effect on incubation period of average nest temperature of naturally incubated Wood Duck nests. Adding the quadratic term increased adjusted r^2 from 0·48 to 0·64, and the parameter estimate (0·76 ± 0·33) for the quadratic term was significantly different from zero ($t = 2{\cdot}29$, $P = 0{\cdot}05$).

We used a randomized complete block design where three temperature treatments were randomly assigned to three eggs from the same nest. Nest was used as the random block effect, and a mixed-model analysis of covariance (ANCOVA) was used for the analysis. Fresh egg mass was used as the covariate because egg and duckling components increase in direct proportion to fresh egg mass (Hepp *et al.* 1987; Kennamer *et al.* 1997).

A series of analyses were performed for each variable. A mixed-model ANCOVA was used across all treatments to test the hypothesis that slopes were equal to zero. If this test was rejected (at least one slope was non-zero), then we tested the hypothesis that slopes for the three treatments were equal. If we failed to reject the hypothesis that slopes were equal, then a common-slope mixed model was used and treatment effects were assessed using adjusted least-squares means. If the initial mixed-model ANCOVA showed that all slopes were not significantly different from zero, then we tested for treatment differences using a mixed-model ANOVA.

We estimated percentage use of lipid, protein and water by Wood Duck embryos during incubation, and used one-way ANOVA to test effects of incubation temperature on percentage nutrient use. Tukey–Kramer tests were used to separate differences between means. Log-likelihood ratio tests were used to test whether hatching success (failed or successful) and ages that embryos died (no development; 1–2 weeks old; 3–4 weeks old) varied among temperature treatments. SAS ver. 8.2 software (SAS Institute 2000) was used for data summaries and statistical analyses.

Results

The average temperature of naturally incubated Wood Duck nests ($n = 12$) at ENWR was 36·2° ± 0·4 °C (range 34·8–37·8 °C), and incubation period averaged 32·2 ± 0·4 days (range 30–35 days). Incubation period declined as average nest temperature increased, and was best fitted with polynomial regression ($F_{2,9} = 10{\cdot}60$, adjusted $r^2 = 0{\cdot}64$, $P = 0{\cdot}004$; Fig. 1).

HATCHING SUCCESS AND INCUBATION PERIOD

We collected three unincubated eggs from each of 48 females ($n = 144$ eggs). The average daily incubation temperature of eggs that were successful and produced ducklings differed by 1·4 °C between adjacent treatments (Table 1). Overall, 62·5% (90 of 144) of eggs hatched, but hatching success differed between treatments ($\chi^2 = 11{\cdot}91$, df = 2, $P = 0{\cdot}003$) and was lowest for eggs incubated at the lowest temperature (Table 1). We placed dead embryos into three age classes (no development; 1–2 weeks; 3–4 weeks). The ages at which embryos died did not differ among treatments ($\chi^2 = 2{\cdot}02$, df = 4, $P = 0{\cdot}73$). Twenty-two per cent (12 of 54) of eggs had no development; 24% (13 of 54) of embryos died at 1–2 weeks; and most (29 of 54) embryos died at 3–4 weeks.

The incubation period of experimental eggs decreased with increasing temperature ($F_{2,59} = 733{\cdot}18$, $P < 0{\cdot}0001$; Table 1), but was not affected by fresh egg mass ($F_{1,51} = 1{\cdot}21$, $P = 0{\cdot}28$). The range of average incubation periods for experimental eggs differed slightly from naturally incubated nests we monitored at ENWR (Fig. 1), but was within the range of values from a much larger sample of nests collected over several years at SRS (Fig. 2). Average incubation period for SRS nests was 31·9 ± 0·1 (range 25–41 days).

NUTRIENT CHANGES AND DUCKLING PHENOTYPE

We estimated lipid, protein and water contents of experimental eggs and computed changes in these components during embryonic development across temperature treatments (Table 2). Eggs lost ≈29% of water during incubation, but changes in water did not differ among treatments ($F_{2,87} = 0{\cdot}26$, $P = 0{\cdot}77$; Table 2). Wood Duck embryos catabolized 34–38% of lipids and 25–33% of protein (Table 2). Percentage change of lipids did not vary among treatments ($F_{2,87} = 2{\cdot}22$,

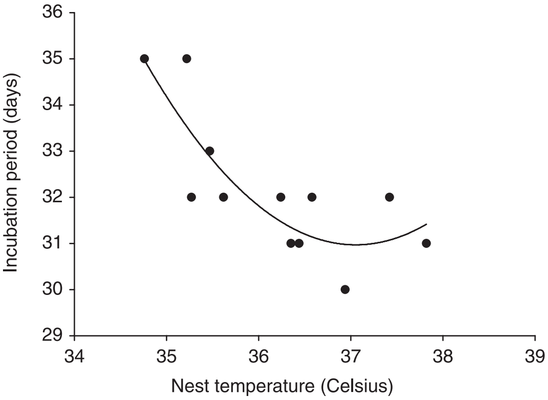

Fig. 1. Relationship between average nest temperature and incubation period in Wood Ducks ($y = 1{\cdot}69{\cdot}7 - 56{\cdot}1x + 0{\cdot}76x^2$, adjusted $r^2 = 0{\cdot}64$).

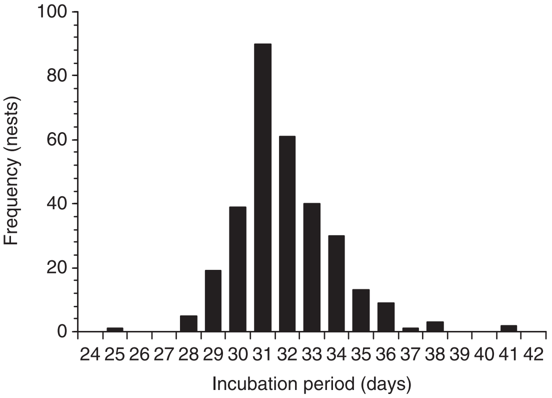

Fig. 2. Frequency distribution of incubation periods of naturally incubated Wood Duck nests ($n = 313$) at the Savannah River Site, 1987–96 and 2003–04.

Table 1. Hatching success, daily incubation temperature (mean ± SE) of eggs that produced ducklings, and incubation period (least-squares mean ± SE) by treatment

Temperature treatment	*n**	Hatching success (%)	Actual incubation temperature (°C)†	Incubation period (days)‡
Low	48	43·8	34·6 ± 0·01	38·8 ± 0·2a
Mid	48	77·1	36·0 ± 0·01	32·0 ± 0·2b
High	48	66·7	37·4 ± 0·01	28·9 ± 0·2c

*Number of eggs incubated.

†Mean daily incubation temperature from start of incubation until the day before eggs pipped.

‡Least-squares means of incubation period followed by different letters are significantly different (*P* ≤ 0·05).

P = 0·12), but embryos incubated at lower temperatures used a greater percentage of protein than those incubated at higher temperatures ($F_{2,87}$ = 30·98, *P* < 0·0001).

In ANCOVAS, wet and dry mass of ducklings and duckling components increased with increasing fresh egg mass (Table 3). Slopes were homogeneous among treatment groups for all variables except wet duckling mass and water, therefore a heterogeneous slope mixed model was used for wet duckling mass, and water and common slope mixed models were used for all other variables. Wet mass of ducklings varied with incubation temperature ($F_{2,68}$ = 3·60, *P* = 0·03) and was lower for eggs incubated at the low incubation temperature compared with eggs incubated at mid- and high temperatures (Table 3). Incubation temperature also affected the dry mass of ducklings ($F_{2,53}$ = 23·99, *P* < 0·0001). Dry duckling mass was greatest for eggs incubated at high temperatures and declined for those incubated at mid- and low temperatures (Table 3).

Composition of Wood Duck neonates also changed with incubation temperature. Water content varied with incubation temperature ($F_{2,64}$ = 4·49, *P* = 0·015), but differences between treatment means were not significant (*P* > 0·05; Table 3). Duckling lipid content did not differ with incubation temperature ($F_{2,52}$ = 2,24, *P* = 0·12), but lean dry mass ($F_{2,57}$ = 40·27, *P* < 0·0001); ash ($F_{2,52}$ = 9·08, *P* = 0·004); and protein ($F_{2,55}$ = 43·65, *P* < 0·0001) of neonates changed with incubation temperature. Lean dry mass and protein were greatest for ducklings that hatched from eggs incubated at the high temperature and declined at mid- and low temperatures (Table 3). Ash content was greater for ducklings incubated at the low temperature and declined for those incubated at mid- and high temperatures (Table 3).

Discussion

We showed that the incubation period of naturally incubated Wood Duck eggs was inversely related to average nest temperature. By experimentally varying the incubation temperature of Wood Duck eggs, we established that incubation period increased with decreasing incubation temperature. Incubation periods of artificially incubated eggs were within the range of incubation periods of naturally incubated Wood Duck nests. We found that incubation temperature influenced not only the incubation period, but also the phenotype of neonates. Wood Duck eggs incubated at low temperatures produced ducklings with reduced body mass and protein, but that were structurally larger than ducklings from eggs incubated at high temperatures. Our study illustrates the importance of egg temperature on development of Wood Duck embryos, and emphasizes the potential importance of incubation on reproductive success in birds.

One weakness of the study is that we used a single incubator for each treatment level, so we were unable to partition potential variation due to incubators. A better design would have replicated treatments across several incubators. It is possible that differences between incubators (e.g. vibration, location), and not temperature *per se*, were responsible for variations in incubation period and neonate phenotype. However, we think this is unlikely because our experimental results were similar to those we observed in naturally incubated nests, and were consistent across years (2003 and 2004). In any event, this weakness should be noted.

The average incubation temperature of avian eggs is variable, and can range between 32 and 38 °C (Webb 1987; Ar & Sidis 2002). Afton & Paulus (1992), in a review of 22 species of ducks and geese, reported a

Table 2. Means (± SE) of Wood Duck egg and neonate composition and predicted changes (%) in lipid, protein and water that occurred during embryonic development at different incubation temperatures*

	Incubation temperature†								
	Low (*n* = 21)			Mid (*n* = 37)			High (*n* = 32)		
Component	Egg (g)	Neonate (g)	Percentage change‡	Egg (g)	Neonate (g)	Percentage change‡	Egg (g)	Neonate (g)	Percentage change‡
Lipid	5·28 (0·08)	3·26 (0·10)	−38·33 (0·02)	5·29 (0·09)	3·45 (0·08)	−34·69 (0·01)	5·25 (0·10)	3·47 (0·47)	−33·64 (0·01)
Protein	6·09 (0·08)	4·09 (0·10)	−33·04a (0·01)	6·11 (0·09)	4·49 (0·08)	−26·51b (0·01)	6·07 (0·10)	4·64 (0·10)	−23·58c (0·01)
Water	26·16 (0·37)	18·53 (0·24)	−29·14 (0·01)	26·22 (0·39)	18·67 (0·26)	−28·67 (0·01)	26·04 (0·46)	18·64 (0·35)	−28·42 (0·01)

*Egg components were estimated from fresh egg mass (g) using data and equations from Hepp *et al.* (1987); see Methods.

†Incubation temperature: low = 34·6; mid- = 36·0; high = 37·4 °C.

‡Mean values of percentage change within rows followed by different letters are significantly different (*P* ≤ 0·05).

Table 3. Least-squares mean (± SE) mass (g) of Wood Duck neonates and their components by incubation temperature

Variable	Incubation temperature* Low	Mid	High	Slope†
Wet duckling	26·69b (0·20)	27·36a (0·15)	27·65a (0·16)	‡
Dry duckling	8·32c (0·10)	8·73b (0·08)	8·96a (0·09)	0·211 (0·016)
Water	18·48 (0·19)	18·63 (0·14)	18·73 (0·15)	‡
Lipid	3·39 (0·08)	3·44 (0·07)	3·53 (0·07)	0·076 (0·013)
Lean dry mass	4·94c (0·05)	5·29b (0·04)	5·46a (0·04)	0·131 (0·008)
Ash	0·85a (0·01)	0·81b (0·01)	0·79b (0·01)	0·015 (0·002)
Protein	4·09c (0·06)	4·48b (0·04)	4·67a (0·05)	0·116 (0·009)

Slopes (b ± SE) are presented from ANCOVAs where fresh egg mass (g) was used as the covariate in analyses of wet and dry duckling mass and component mass. Within rows, least-squares means followed by different letters are significantly different ($P \le 0{\cdot}05$).

*Incubation temperature: low = 34·6; mid- = 36·0; high = 37·4 °C.

†Unless noted, slopes did not differ ($P > 0{\cdot}05$) among incubation temperatures; common slope mixed models were used to test treatment effects.

‡Slopes for wet duckling mass and water were not homogeneous ($P < 0{\cdot}05$). Wet duckling: low (0·467 ± 0·077); mid- (0·530 ± 0·047); high (0·670 ± 0·034); water: low (0·253 ± 0·076); mid- (0·347 ± 0·039); high (0·464 ± 0·033).

mean egg temperature of 35·6 ± 0·4 (range 31·3–39·6 °C). The incubation temperatures we used for experimental eggs were well within the range of egg temperatures reported for naturally incubated waterfowl nests.

Under natural conditions, egg temperature can be influenced by behaviour of incubating parents, characteristics of nests, developing embryos, and environmental conditions such as ambient temperature, wind speed and solar radiation (Deeming 2002b). Maintenance of proper egg temperature during incubation is important because egg temperature can influence the length of the incubation period, as we have shown in Wood Ducks. Mound-builders (Megapodiidae) exhibit striking intraspecific variability in incubation periods (e.g. the Mallee Fowl, *Leipoa ocellata*, 44–99 days), which has been attributed to differences in mound temperature (Booth & Jones 2002). In nests where male European Starlings (*Sturnus vulgaris*) assisted females with incubation, eggs spent more time at higher temperatures and had shorter incubation periods than eggs in nests where females incubated alone (Reid, Monaghan & Ruxton 2002b). Similarly, eggs of Brown-headed Cowbirds (*Molothrus ater*) hatched earlier when incubated at higher temperatures (Strausberger 1998).

Lengthy incubation periods can be costly because they result in greater exposure of nests to predators (Reid, Monaghan & Nager 2002a). Tombre & Erikstad (1996) manipulated the incubation period of Barnacle Geese (*Branta leucopsis*), and reported that females with extended incubation periods experienced greater egg loss to predators than females with shortened incubation periods. Predation is a major cause of nest failure in birds and, because of its importance to reproductive success, has influenced a variety of life-history traits (Arnold, Rohwer & Armstrong 1987; Martin 1992; Conway & Martin 2000). Risk of predation should favour short incubation periods.

The effect of incubation microclimate on neonate phenotype remains relatively unexplored in birds, but has been studied extensively in reptiles (Cagle *et al.* 1993; Janzen 1993; Shine, Elphick & Harlow 1997; Burger 1998; Booth *et al.* 2000; Ashmore & Janzen 2003; Brown & Shine 2004). Both thermal and hydric characteristics of nest sites can be important to reptiles. In some species, wet incubation environments produced larger neonates than dry environments, and large hatchlings survived better than small hatchlings (Janzen 1993; Brown & Shine 2004). Embryos of some reptiles incubated at low temperature had longer incubation periods and used more energy than those incubated at high temperature (Angilletta, Winters & Dunham 2000; Booth 2000).

In birds, eggs of Mallee Fowl incubated at low temperatures had longer incubation periods and used 74% more energy than eggs incubated at high temperatures (Booth 1987). Eggs of Tree Swallows (*Tachycineta bicolor*) and European Starlings incubated at reduced temperatures also took longer to hatch and produced smaller neonates (Lombardo *et al.* 1995; Reid *et al.* 2002b). More recently, Göth & Booth (2005) reported that incubation temperature influenced sex ratio and body mass of neonate Australian Brush-turkeys (*Alectura lathami*). Temperature-sensitive embryo mortality resulted in more male Brush-turkeys hatching at low temperatures (31 °C) and more females hatching at high temperatures (36 °C); body mass of neonates was inversely related to incubation temperature (Göth & Booth 2005). In our study, low incubation temperatures lengthened the incubation period of Wood Ducks and produced neonates with reduced body and protein masses. Metabolic rates of avian embryos decline with decreasing incubation temperature, but long incubation periods associated with low incubation temperature increase maintenance costs and total energy expended by developing embryos (Vleck, Vleck & Seymour 1984; Vleck & Vleck 1996).

We expected that Wood Duck neonates with longer incubation periods would use more lipids and hatch with reduced lipid mass, compared with neonates that hatched earlier. Wet and dry body masses declined with increases in incubation period, and although the lipid mass of neonates decreased with increasing incubation period, differences were not significant. Instead, embryos with long incubation periods catabolized more egg protein and hatched with reduced protein mass. If the total energy expenditure of Wood Duck embryos increased with increasing incubation period, then embryos used a combination of protein and lipid to satisfy higher energetic costs. Small ducklings with reduced protein mass may encounter difficulties after hatching because their ability to develop and maintain homeothermy will be reduced compared with large ducklings that have greater protein mass (Rhymer 1988; Visser & Ricklefs 1995). Skeletal muscles, for example, provide an important source of heat production in young birds exposed to cold temperatures (Olson 1994). Loss of muscle mass might also diminish locomotor performance.

Conditions experienced by neonates during early development can affect subsequent survival and reproduction (Lindström 1999; Metcalfe & Monaghan 2001; Gorman & Nager 2004). However, there is a lack of integrated studies of birds that first assess effects of incubation microclimate on neonate phenotype, and then determine whether phenotypic differences influence fitness. In precocial species, size of neonates is often positively related to survival (Dawson & Clark 1996; Pelayo & Clark 2003); growth rate (Anderson & Alisauskas 2002); ability to thermoregulate (Rhymer 1988); and locomotor performance (Anderson & Alisauskas 2001; Göth & Evans 2004). Therefore the effects of incubation environment on variation in offspring phenotype may have important consequences for neonate survival and subsequent reproduction.

Incubation can be an important reproductive cost in birds (Reid *et al*. 2002a). Incubating adults face trade-offs between self-maintenance and providing the proper environment for embryo development; constraints may intensify for uniparental incubators. Most studies have emphasized the potential costs of incubation on parents (such as survival and physical condition of incubating adults), but here we show decisions made by incubating adults that influence egg temperature may modify neonate phenotype. Future investigations of incubation as a reproductive cost in birds must include how changes to the incubation environment affect both parents and offspring.

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References

Afton, A.D. & Paulus, S.L. (1992) Incubation and brood care. In: *Ecology and Management of Breeding Waterfowl* (eds B.D.J. Batt, A.D. Afton, M.G. Anderson *et al.*), pp. 62–108. University of Minnesota Press, Minneapolis, MN, USA.

Aldrich, T.W. & Raveling, D.G. (1983) Effects of experience and body weight on incubation behavior of Canada geese. *Auk* **100**, 670–679.

Anderson, V.R. & Alisauskas, R.T. (2001) Egg size, body size, locomotion, and feeding performance in captive king eider ducklings. *Condor* **103**, 195–199.

Anderson, V.R. & Alisauskas, R.T. (2002) Composition and growth of king eider ducklings in relation to egg size. *Auk* **119**, 62–70.

Angilletta, M.J., Winters, R.S. & Dunham, A.E. (2000) Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* **81**, 2957–2968.

Ar, A. & Sidis, Y. (2002) Nest microclimate during incubation. In: *Avian Incubation: Behavior, Environment, and Evolution* (ed. D.C. Deeming), pp. 143–160. Oxford University Press, New York.

Arnold, T.W., Rohwer, F.C. & Armstrong, T. (1987) Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. *American Naturalist* **130**, 643–653.

Ashmore, G.M. & Janzen, F.J. (2003) Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182–188.

Badyaev, A.V., Hill, G.E., Beck, M.L. *et al.* (2002) Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* **295**, 316–318.

Bellrose, F.C. & Holm, D.J. (1994) *Ecology and Management of the Wood Duck*. Stackpole Books, Mechanicsburg, PA, USA.

Bernardo, J. (1996) Maternal effects in animal ecology. *American Zoologist* **36**, 83–105.

Bolton, M. (1991) Determinants of chick survival in the lesser black-backed gull: relative contributions of egg size and parental quality. *Journal of Animal Ecology* **60**, 949–960.

Booth, D.T. (1987) Effect of temperature on development of mallee fowl *Leipoa ocellata* eggs. *Physiological Zoology* **60**, 437–445.

Booth, D.T. (2000) Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: effects on pattern of oxygen consumption and hatchling morphology. *Australian Journal of Zoology* **48**, 369–378.

Booth, D.T. & Jones, D.N. (2002) Underground nesting in megapodes. In: *Avian Incubation: Behavior, Environment, and Evolution* (ed. D.C. Deeming), pp. 192–206. Oxford University Press, New York.

Booth, D.T., Thompson, M.B. & Herring, S. (2000) How incubation temperature influences the physiology and growth of embryonic lizards. *Journal of Comparative Physiology B* **170**, 269–276.

Brown, G.P. & Shine, R. (2004) Maternal nest-site choice and offspring fitness in a tropical snake (*Tropidonophis mairii*), Colubridae. *Ecology* **85**, 1627–1634.

Burger, J. (1998) Effects of incubation temperature on hatchling pine snakes: implications for survival. *Behavioral Ecology and Sociobiology* **43**, 11–18.

Cagle, K.D., Packard, G.C., Miller, K. & Packard, M.J. (1993) Effects of microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Functional Ecology* **7**, 653–660.

Carey, C. (1980) The ecology of avian incubation. *Bioscience* **30**, 819–824.

Clawson, R.L., Hartman, G.W. & Fredrickson, L.H. (1979) Dump nesting in a Missouri wood duck population. *Journal of Wildlife Management* **43**, 347–355.

Conway, C.J. & Martin, T.E. (2000) Evolution of passerine incubation behavior: influence of food, temperature, and nest predation. *Evolution* **54**, 670–685.

Dawson, R.D. & Clark, R.G. (1996) Effects of variation in egg size and hatching date on survival of lesser scaup *Aythya affinis* ducklings. *Ibis* **138**, 693–699.

Deeming, D.C. (2002a) Importance and evolution of incubation in avian reproduction. In: *Avian Incubation: Behavior, Environment, and Evolution* (ed. D.C. Deeming), pp. 1–7. Oxford University Press, New York.

Deeming, D.C., ed. (2002b) *Avian Incubation: Behavior, Environment, and Evolution*. Oxford University Press, New York.

Deeming, D.C. (2002c) Behavior patterns during incubation. In: *Avian Incubation: Behavior, Environment, and Evolution* (ed. D.C. Deeming), pp. 63–87. Oxford University Press, New York.

Deeming, D.C. & Ferguson, M.W.J. (1991) Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles* (eds D.C. Deeming & M.W.J. Ferguson), pp. 147–171. Cambridge University Press, New York.

Dobush, G.R., Ankney, C.D. & Krementz, D.G. (1985) The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. *Canadian Journal of Zoology* **63**, 1917–1920.

Drobney, R.D. (1980) Reproductive bioenergetics of wood ducks. *Auk* **97**, 480–490.

Folk, T.H. & Hepp, G.R. (2003) Effects of habitat use and movement patterns on incubation behavior of female wood ducks (*Aix sponsa*) in southeast Alabama. *Auk* **120**, 1159–1167.

Gorman, H.E. & Nager, R.G. (2004) Prenatal developmental conditions have long-term effects on offspring fecundity. *Proceeding of the Royal Society of London, B* **271**, 1923–1928.

Göth, A. & Booth, D.T. (2005) Temperature-dependent sex ratio in a bird. *Biology Letters* **1**, 31–33.

Göth, A. & Evans, C.S. (2004) Egg size predicts motor performance and postnatal weight gain of Australian brush-turkey (*Alectura lathami*) hatchlings. *Canadian Journal of Zoology* **82**, 972–979.

Hanson, H.C. (1954) Criteria of age of incubated mallard, wood duck, and bob-white quail eggs. *Auk* **71**, 267–272.

Hepp, G.R. (2004) Onset of incubation in wood ducks. *Condor* **106**, 182–186.

Hepp, G.R., Stangohr, D.J., Baker, L.A. & Kennamer, R.A. (1987) Factors affecting variation in the egg and duckling components of wood ducks. *Auk* **104**, 435–443.

Hepp, G.R., Kennamer, R.A., Harvey, I.V. & W.F. (1989) Recruitment and natal philopatry of wood ducks. *Ecology* **70**, 897–903.

Janzen, F.J. (1993) An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* **74**, 332–341.

Kennamer, R.A. & Hepp, G.R. (2000) Integration of research with long-term monitoring: breeding wood ducks on the Savannah River Site. *Studies in Avian Biology* **21**, 39–49.

Kennamer, R.A., Harvey, I.V., W.F. & Hepp, G.R. (1990) Embryonic development and nest attentiveness of wood ducks during egg laying. *Condor* **92**, 587–592.

Kennamer, R.A. Alsum, S.K. & Colwell, S.V. (1997) Composition of wood duck eggs in relation to egg size, laying sequence, and skipped days of laying. *Auk* **114**, 479–487.

Lindström, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology and Evolution* **14**, 343–348.

Lombardo, M.P. Bosman, R.M. Faro, C.A. Houtteman, S.G. & Kluisza, T.S. (1995) Effect of feathers as nest insulation on incubation behavior and reproductive performance of tree swallows (*Tachycineta bicolor*). *Auk* **112**, 973–981.

Manlove, C.A. & Hepp, G.R. (1998) Effects of mate removal on incubation behavior and reproductive success of female wood ducks. *Condor* **100**, 688–693.

Manlove, C.A. & Hepp, G.R. (2000) Patterns of nest attendance in female wood ducks. *Condor* **102**, 286–291.

Martin, T.E. (1992) Interaction of nest predation and food limitation in reproductive strategies. *Current Ornithology* **9**, 163–197.

Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution* **16**, 254–260.

Mousseau, T.A. & Fox, C.W. (1998) The adaptive significance of maternal effects. *Trends in Ecology and Evolution* **13**, 403–407.

Olson, J.M. (1994) The ontogeny of shivering thermogenesis in the red-winged blackbird (*Agelaius phoeniceus*). *Journal of Experimental Biology* **191**, 59–88.

Pelayo, J.T. & Clark, R.G. (2003) Consequences of egg size for offspring survival: a cross-fostering experiment in ruddy ducks (*Oxyura jamaicensis*). *Auk* **120**, 384–393.

Price, T. (1998) Maternal and paternal effects in birds. In: *Maternal Effects as Adaptations* (eds T.A. Mousseau & C.W.J. Fox), pp. 202–226. Oxford University Press, New York.

Reid, J.M. Monaghan, P. & Nager, R.G. (2002a) Incubation and the costs of reproduction. In: *Avian Incubation: Behavior, Environment, and Evolution* (ed. D.C. Deeming), pp. 314–325. Oxford University Press, New York.

Reid, J.M. Monaghan, P. & Ruxton, G.D. (2002b) Males matter: the occurrence and consequences of male incubation in starlings (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology* **51**, 255–261.

Rhymer, J.M. (1988) The effect of egg size variability on thermoregulation of mallard (*Anas platyrhynchos*) offspring and its implications for survival. *Oecologia* **75**, 20–24.

SAS Institute Inc. (2000) *SAS Onlinedoc*, Version 8. SAS Institute, Cary, NC, USA.

Semel, B. & Sherman, P.W. (1986) Dynamics of nest parasitism in wood ducks. *Auk* **103**, 813–816.

Shine, R., Elphick, M.J. & Harlow, P.S. (1997) The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**, 2559–2568.

Strausberger, B.M. (1998) Temperature, egg mass, and incubation time: a comparison of brown-headed cowbirds and red-winged blackbirds. *Auk* **115**, 843–850.

Tombre, I.M. & Erikstad, K.E. (1996) An experimental study of incubation effort in high-Arctic barnacle geese. *Journal of Animal Ecology* **65**, 325–331.

Visser, G.H. & Ricklefs, R.E. (1995) Relationship between body composition and homeothermy in neonates of precocial and semiprecocial birds. *Auk* **112**, 192–200.

Vleck, C.M. & Vleck, D. (1996) Embryonic energetics. In: *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 417–460. Chapman & Hall, New York.

Vleck, C.M., Vleck, D. & Seymour, R.S. (1984) Energetics of embryonic development in the megapode birds, mallee fowl *Leipoa ocellata* and brush turkey *Alectura lathami*. *Physiological Zoology* **57**, 444–456.

Webb, D.R. (1987) Thermal tolerance of avian embryos: a review. *Condor* **89**, 874–898.

Weller, M.W. (1956) A simple field candler for waterfowl eggs. *Journal of Wildlife Management* **20**, 111–113.

White, F.N. & Kinney, J.L. (1974) Avian incubation. *Science* **186**, 107–115.

Williams, T.D. (1994) Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biological Review* **68**, 35–59.

Zicus, M.C., Hennes, S.K. & Riggs, M.R. (1995) Common goldeneye nest attendance patterns. *Condor* **97**, 461–472.

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