Incubation Conditions Are More Important in Determining Early Thermoregulatory Ability than Posthatch Resource Conditions in a Precocial Bird

S. E. DuRant[1,2]
W. A. Hopkins[1,*]
A. W. Carter[1]
C. M. Stachowiak[1]
G. R. Hepp[3]
[1]Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, Virginia 24061; [2]Department of Biology, Tufts University, Medford, Massachusetts 02155; [3]School of Forestry and Wildlife Sciences, Auburn University, Auburn, Alabama 36849

Accepted 4/14/2013; Electronically Published 5/31/2013

ABSTRACT

Recent research in birds suggests that investing in incubation is one mechanism by which parents can enhance the phenotype of their offspring. Posthatch environmental conditions can also shape an individual's phenotype, and it is thus possible for pre- and posthatch conditions to have interactive effects on an individual's phenotype. In this study, we examined the individual and interactive effects of prehatch incubation temperature and posthatch food availability on growth, food consumption, and thermoregulatory ability in wood duck (*Aix sponsa*) ducklings. Eggs were incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C), and then ducklings were reared on an either ad lib. or time-restricted diet for 12 d after hatching. We found that food availability influenced duckling growth, with the slowest growth occurring in ducklings fed the restricted diet. Incubation temperature also interacted with food conditions to influence duckling growth: ducklings fed ad lib. from the lowest incubation temperature grew slower than ducklings fed ad lib. from the higher incubation temperatures. Most importantly, we found that the improvement in a duckling's ability to maintain body temperature in the face of a thermal challenge was influenced by embryonic incubation temperature but not feeding conditions. Ducklings from the highest incubation temperature experienced the greatest improvement in thermoregulatory performance with age. Our findings suggest that the prehatch environment is more important than posthatch resource conditions in determining some physiological functions and underscores the important role that incubation temperature plays in determining offspring phenotype in birds.

* Corresponding author; e-mail: hopkinsw@vt.edu.

Physiological and Biochemical Zoology 86(4):410–420. 2013. 1522-2152/2013/8604-2083$15.00. DOI: 10.1086/671128

Introduction

Parents can provide fitness advantages to their offspring by making resource investments (e.g., time, energy) in their young early in development. For instance, in some oviparous vertebrates, young hatching from larger eggs tend to have heavier body mass, higher survival, and better competitive abilities than conspecifics hatched from smaller eggs (Rhymer 1988; Williams 1994; Cox et al. 1998; Einum and Fleming 2000; but see Congdon et al. 1999). In birds, much research on early resource investments in young has focused on egg size and, to a lesser extent, the allocation of important resources—such as hormones and carotenoids—to eggs and the implications of these parental effects for embryonic developmental rates, offspring size at hatching, competitive abilities, growth rates, and immune responses (Williams 1994; Saino et al. 2003; Groothuis et al. 2005; Eising et al. 2006; Groothuis and Schwabl 2008; Biard et al. 2009). Although not widely studied in birds, parental investments in incubation, which influence incubation temperature, appear to be crucial in determining phenotypes of avian offspring as well. Incubation temperature can affect a suite of phenotypic traits important for future development and survival, including offspring size at hatching, growth, body condition, locomotor performance, thermoregulation, metabolic rate, immune responses, and stress endocrinology (Hepp et al. 2006; DuRant et al. 2010, 2012*a*, 2012*b*; Hopkins et al. 2011; Nilsson and Nord 2011). Furthermore, a recent study demonstrated that females incubated at lower temperatures have lower survival and reproductive success in the wild than females incubated at higher temperatures (Hepp and Kennamer 2012). Thus, early investments made by parents during incubation could ultimately increase both their fitness and the fitness of their offspring by generating nest temperatures that produce young with high-quality phenotypes.

Environmental conditions after hatching can also affect avian offspring traits important for survival, and food resources are a critical component of the posthatch environment. Both food quantity and quality can influence growth rates, fledging success, survival, hormone secretion, and cognitive development in young birds (Uttley et al. 1994; Cox et al. 1998; Lepage et

al. 1998; Gunnarsson et al. 2004; Kitaysky et al. 2006; Romano et al. 2006). For example, lipid content of fish fed to nestling red-legged kittiwakes (*Rissa brevorostris*) can affect their learning ability, with subsequent implications for spatial memory after fledging, and could ultimately contribute to reduced recruitment in Alaskan populations (Kitaysky et al. 2006). In addition, a study in snow geese (*Chen caerulescens atlantica*) revealed that goslings had higher growth rates in years of greater food abundance (Lepage et al. 1998). In altricial birds, parents often increase foraging effort to compensate for low food abundance, allowing their young to grow at similar rates in both food-rich and food-poor years (Dunn and Hannon 1992; Tremblay et al. 2005; Cucco and Malacarne 2009). In contrast, for many precocial species (e.g., waterfowl), parents may have less influence over resource conditions posthatch because their offspring must acquire much of their own resources after hatching. Thus, early parental investments in precocial offspring, such as energy allocation to eggs and maintenance of optimal incubation conditions, may be crucial for offspring posthatch growth and survival. Research has yet to determine how prehatch developmental experiences such as incubation temperature interact with differing levels of food availability in the posthatch environment, but there is evidence that interactions at other stages of ontogeny exist (Monaghan 2008). For instance, in red-billed choughs (*Pyrrhocorax pyrrhocorax*), both the quality of the environment where chicks were reared and the quality of the environment in which they later nested determined their breeding success as adults (Reid et al. 2006). This study indicated that the negative consequences of the natal environment may be context dependent and be ameliorated by better environmental conditions experienced later in life.

In this study, we tested the individual and interactive effects of prehatch incubation temperature and posthatch resource conditions on food consumption, growth, and thermoregulation of wood duck (*Aix sponsa*) ducklings. To accomplish this, we incubated eggs collected from the field at three ecologically relevant temperatures (35.0°, 35.9°, and 37.0°C) and then reared offspring with either ad lib. or restricted access to food. For many precocial avian offspring, one of the greatest threats to survival is hypothermia (Talent et al. 1983; Bellrose and Holm 1994; Mauser et al. 1994; Korschgen et al. 1996). Wood ducks begin hatching in late winter and early spring, when temperatures can drop below freezing. Although protein content of the leg and pectoral muscles is important for shivering thermogenesis, larger body size can also provide thermoregulatory advantages by reducing surface area to volume ratio and associated heat loss and mass-specific energetic demands (Visser 1998). Because previous research demonstrated that low incubation temperatures negatively affected duckling protein content, duckling growth up to 20 d, and thermoregulation (Hepp et al. 2006; DuRant et al. 2010, 2012*a*, 2012*b*), we predicted that ducklings hatched from eggs incubated at the lowest temperature would exhibit slower growth and poorer thermoregulatory ability compared with ducklings from eggs incubated at higher temperatures. We also predicted that ducklings reared on a food-restricted diet would eat less, grow slower, and have poorer thermoregulatory ability than ducklings reared on an ad lib. diet. Last, we predicted that incubation temperature and feeding conditions would have an additive effect on duckling growth and thermoregulation, such that the deficiencies in growth and thermoregulatory performance among treatment combinations would be most pronounced in ducklings from the lowest incubation temperature and fed the restricted diet.

Material and Methods

Study Species

The wood duck is an abundant species of waterfowl that inhabits a variety of lentic and lotic habitats near timber stands or those that provide inundated/overhanging shoreline vegetation (Bellrose and Holm 1994). Egg laying begins in late January in populations at lower latitudes and in early April at higher latitudes of the wood duck's range. Wood ducks are secondary cavity nesters and readily use nest boxes; thus, eggs can be collected easily throughout the entire breeding season (Hepp and Bellrose 1995). Their clutch size averages 12 eggs, and only the female incubates the eggs. Females begin night incubation when ~75% of the clutch is laid, and constant incubation begins at the end of clutch completion (Hepp and Bellrose 1995). Incubation period varies from 27 to 37 d (Hepp and Bellrose 1995), and many females in our population produce multiple clutches in a season. On the basis of data from temperature loggers in nests for the duration of incubation, average incubation temperature ranges from 34.8° to 37.5°C (Manlove and Hepp 2000; Hepp et al 2006). Wood ducks hatch synchronously, and ducklings generally leave the nest box within 24 h of hatching (Bellrose and Holm 1994). On hatching, ducklings are exposed to cold ambient temperatures, with minimums between 0.0° and 5.6°C (Bellrose and Holm 1994). Ducklings normally feed during the early morning and evening, with most inactivity occurring midday (Bellrose and Holm 1994). Ducklings typically reach adult size and are able to fly 55–60 d posthatching (Bellrose and Holm 1994).

Study Site

Our study site consists of nest boxes ($n = 92$) located on 12 isolated wetlands scattered across the Department of Energy's Savannah River Site in South Carolina. The nest boxes at these sites have been available for use by wood ducks for more than 20 yr and have been continuously monitored.

Egg Collection and Incubation

From February to April 2011, we collected freshly laid eggs daily from nest boxes (every morning shortly after egg laying occurred) in which a nest was initiated before the onset of night incubation. Every 4 d, we transported eggs to Virginia Tech. Avian embryos do not develop when maintained below 24°–27°C (White and Kinney 1974), and holding duck eggs at these temperatures for up to 10 d does not affect hatchability

(Walls et al. 2011). We artificially incubated eggs in Grumbach incubators (model BSS 160) at three average temperatures (35.0°, 35.9°, and 37.0° ± 0.1°C) within the range of naturally incubated wood duck nest temperatures (Manlove and Hepp 2000; Hepp et al 2006). All eggs were incubated at ~60% humidity. Two preprogrammed cool-down periods occurred each day (~3°C reduction in mean temperature for 75 min at 0815 and 1830 hours) to simulate daily feeding recesses taken by mothers during incubation (Manlove and Hepp 2000). Eggs were randomly assigned to an incubation temperature, but we stratified our assignments by clutch to ensure that eggs from the same clutch were evenly dispersed among temperatures. Egg incubation and all experimental procedures were conducted in labs on the Virginia Tech campus.

Duck Husbandry

After hatching, ducklings were maintained in pairs in 46 × 32 × 24.5-cm plastic cages (2 ducklings/cage; both ducklings were from the same incubation temperature) in a temperature-controlled environmental chamber (28°C, 14L : 10D photoperiod). A 50-W infrared light bulb suspended above each cage provided additional warmth to ducklings and created a thermal gradient from 30° to 37°C within each cage. Ducklings had constant access to water and were fed Dumor Chick Starter/Grower 20% protein. All animal husbandry followed Institutional Animal Care and Use Committee–approved procedures, as outlined by DuRant et al. (2010, 2012*a*, 2012*b*).

Experimental Design and Time Line

We tested for the individual and interactive effects of incubation temperature and posthatch nutritional conditions on duckling thermoregulation, using a 3 × 2 factorial design that included three incubation temperatures (35.0°, 35.9°, and 37.0°C) and two feeding regimes, ad lib. and restricted (n = 12–19 ducklings/incubation temperature × feeding treatment; total = 94 ducklings). To avoid confounding maternal effects (and pseudoreplication), we included only one duckling per clutch in an incubation temperature × feeding regime treatment, using stratified random sampling. We thermally challenged ducklings (1–9 ducklings/trial) at 1 and 4 d posthatch. Following the protocol of Rhymer (1988) and DuRant et al. (2012*b*), ducklings were not fed until after the first thermal trial (1 d posthatch). Ducklings catabolize yolk during the first days after hatching and do not begin feeding in the laboratory until 24–48 h after hatching (S. E. DuRant, personal observation).

Duckling Feeding and Growth

We carefully quantified food consumption of ducklings to verify that our technique of manipulating resource abundance was successful. We gave ad lib. ducklings preweighed (on the basis of dry weight; all food was dried in a drying oven) food every morning at ~0930 hours and removed food 24 h later. We then removed feces from food dishes, dried the food again, weighed the remaining food, and calculated the grams (to nearest 0.01 g) of food consumed. Because ducklings were housed in pairs, we divided the amount of food consumed in each cage by two to provide an estimate of food consumption for each duckling. Ducklings had to be housed in pairs because they are very social and often do not eat when housed alone. While ducklings in the ad lib. treatment had constant access to food, ducklings in the food-restricted treatment had access to food two times a day, from 0600 to 0900 hours and from 1700 to 2000 hours. Thus, food-restricted ducklings were not restricted in the amount of food offered but in the amount of time available to feed. We chose these feeding times because ducklings in the wild typically feed for two intervals during the day, in the morning and in the afternoon (Bellrose and Holm 1994). Food rations for all ducklings in all treatments started at 20 g/d per duck and increased over time on the basis of duckling feeding rates such that each pair of ducks always received more food than they could consume in a feeding interval (restricted ducklings) or a 24-h interval (ad lib. ducklings). We measured duckling mass every morning for 12 d and duckling tarsus length on 0, 1, 4, 8, and 12 d posthatch in order to compare duckling growth and body condition.

Duckling Thermoregulation

We examined the thermoregulatory capacity of ducklings by conducting a cold-challenge experiment similar to that described by Rhymer (1988) and DuRant et al. (2012*b*). We thermally challenged ducklings in all treatment groups at 1 and 4 d posthatch. The thermal challenge consisted of exposure to 10.0°C for 1 h (Caldwell 1972; Rhymer 1988; DuRant et al. 2012*b*). Our challenge temperature was within the range of temperatures experienced by ducklings in the field in South Carolina and is likely below the thermoneutral zone for 1-d-old wood duck ducklings (DuRant et al. 2012*b*). Although the precise lower critical temperature 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 d posthatch is 32°C (Koskimies and Lahti 1964). Wood ducks' lower critical temperature is probably similar because their body mass falls between that of Eurasian teal (body mass at 1 d old is ~16.8 g) and mallards (body mass at 1 d old is ~28.8 g; Koskimies and Lahti 1964).

For each thermal challenge, we acclimated ducklings in individual 1-L containers within an environmental chamber for 1 h at a thermoneutral temperature (36°C). After acclimation, we measured body temperature of ducklings, using a cloacal thermometer (Scultheis T6000; Miller and Weber), then lowered the environmental chamber temperature to 10°C over a period of ~5 min. After 1 h at 10°C, we measured cloacal temperature again. Body temperature was measured within 1 min to minimize handling effects. We then calculated the percent change in a duckling's body temperature from before to after the thermal challenge and used percent change in body temperature in statistical analyses to account for individual differences in body temperature at thermoneutrality. However,

Table 1: Least squares means (±1 SE) of three different measures of duckling size for all ducklings incubated in 2011

	Incubation temperature			
	35.0°C ($n = 101$)	35.9°C ($n = 99$)	37.0°C ($n = 82$)	P
Mass (g)	26.9 (.16)	27.0 (.16)	27.6 (.17)	.01
Tarsus (mm)	19.1 (.08)	19.0 (.08)	18.2 (.08)	<.001
Culmen (mm)	15.2 (.15)	15.3 (.15)	15.2 (.16)	.75
Body condition	26.6 (.26)	26.8 (.26)	28.3 (.29)	<.001

Note. Mass, tarsus, and culmen are corrected for egg size, whereas body condition represents body mass corrected for tarsus length. Ducklings hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, and 37.0°C).

analyses of absolute change in a duckling's body temperature yielded the same results. To assess each duckling's improvement in thermoregulatory ability as they aged, we also calculated the difference in an individual's percent change in body temperature on day 4 relative to day 1 (expressed as change in percentage points).

Statistical Analyses

All statistical analyses were run in SAS 9.1 (SAS Institute, Cary, NC) or Microsoft Excel, and statistical significance was recognized at $\alpha < 0.05$. 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 models.

Since experimental treatment was randomly assigned posthatching, we determined whether incubation temperature influenced hatching success and incubation period of all eggs incubated in 2011 ($N = 384$) even though only a portion of the hatchlings produced by these eggs was used in this study. Hatching success was modeled as binary data, with incubation temperature as the main effect and clutch origin as a random effect (SAS proc NLMIXED). Incubation period was analyzed using ANOVA (SAS proc mixed), again with clutch origin included as a random effect. We also investigated the effects of incubation temperature on duckling size at hatching (mass, tarsus length, and culmen length) using a series of ANCOVAs. Models included egg mass as a covariate and clutch origin as a random effect. Hatchling body condition was analyzed using ANCOVA by comparing hatchling mass among treatments, with tarsus length as a covariate and clutch as a random effect.

To determine the effects of incubation temperature and feeding regime on food consumption, duckling growth, body condition, and percent change in a duckling's body temperature during thermal trials 1 and 4 d posthatch for the ducklings ($N = 94$) used in this study, we conducted four separate repeated-measures ANOVAs using either SAS proc mixed or proc glm, depending on whether there were missing data points. We used autoregressive and compound symmetry covariance structures for mixed models on the basis of which best fit the data. All models included feeding regime, duckling age, and incubation temperature as main effects as well as all two-way and three-way interactions. To estimate body condition, we first regressed mass against tarsus length at 0, 1, 4, 8, and 12 d posthatch. The residuals produced at each date were used as the dependent variable in the model. To account for mild violations of the equal variance assumption, we used Satterwaithe's method to estimate degrees of freedom in our food consumption model. In the body temperature model, we included body mass as a covariate, and we rank transformed percent change in body temperature to meet assumptions of the model. Because our study was designed to examine interactions between the prehatch and posthatch environment over time, for all models the full model (included all interactions) is also the final model. However, we also ran models that excluded all insignificant interactions, and these models always generated similar results.

Last, to determine the effects of incubation temperature and feeding regime on the improvement in a duckling's thermoregulatory ability from 1 to 4 d posthatch, we used a two-way ANCOVA (SAS proc mixed). We used change in percentage points from 1 to 4 d posthatch as our response variable in the model. To account for effects of body size on thermoregulatory ability, we included mass at 1 d posthatch as a covariate.

Results

Hatching success of wood duck eggs ranged from 69% to 77% and was similar across all incubation temperatures ($P = 0.265$). Incubation period differed significantly among incubation temperatures ($F_{2,236} = 570$, $P < 0.001$), with the longest incubation period occurring at the lowest temperature and the shortest incubation period occurring at the highest incubation temperature (35.0°C: 37.2 ± 0.1 d; 35.9°C: 34.6 ± 0.1 d; 37.0°C: 30.8 ± 0.1 d). In addition, ducklings incubated at the highest temperature had higher body mass and body condition at hatching but smaller tarsus length than ducklings incubated at the medium and low incubation temperatures (table 1; mass: $F_{2,229} = 4.53$, $P = 0.012$; tarsus: $F_{2,229} = 47.05$, $P < 0.001$; body condition: $F_{2,229} = 15.12$, $P < 0.001$). There was no difference in culmen length among incubation temperatures (table 1; culmen length: $P = 0.75$). Egg mass had a significant positive

Table 2: Morphometrics of the random subset of ducklings from 2011 used in the current feeding study: least squares means (±1 SE) of four different measures of duckling size

	Incubation temperature			
	35.0°C ($n = 35$)	35.9°C ($n = 32$)	37.0°C ($n = 27$)	P
Mass (g)	27.1 (.27)	27.5 (.28)	27.4 (.30)	.51
Tarsus (mm)	19.2 (.12)	19.1 (.12)	18.4 (.13)	<.001
Culmen (mm)	15.3 (.12)	15.5 (.13)	15.5 (.14)	.30
Body condition	26.8 (.41)	27.5 (.41)	27.2 (.52)	.10

Note. Mass, tarsus, and culmen are corrected for egg size, whereas body condition represents body mass corrected for tarsus length. Ducklings hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, and 37.0°C).

Table 3: Results (P values) of four repeated-measures ANOVAs conducted to determine the effects of incubation temperature and feeding regime (ad lib. or restricted) over time on food consumption, growth, body condition, and body temperature of ducklings hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, and 37.0°C)

	Food consumption	Growth	Body condition	Body temperature
Incubation temperature	.12	.25	.003	.67
Feeding regime	<.001	<.001	<.001	.09
Age	<.001	<.001	1.00	.35
Incubation temperature × feeding regime	.03	.20	.41	.40
Incubation temperature × age	.93	.42	.18	.001
Feeding regime × age	<.001	<.001	<.001	.80
Incubation temperature × feeding regime × age	.79	.001	.29	.82
Body mass (covariate)	NA	NA	NA	.002

Note. NA, not applicable.

influence on all measures of duckling size (all cases: $F \geq 18.79$, $P < 0.001$). For the subset of ducklings used in the food manipulation experiment, body size data followed similar trends, except that body mass and condition did not differ significantly among incubation temperatures (table 2; tarsus: $F_{2,90} = 10.52$, $P < 0.001$; culmen: $P = 0.30$; mass: $P = 0.51$; body condition: $P = 0.10$).

As expected, ducklings fed an ad lib. diet consumed significantly more food than ducklings fed a restricted diet (table 3; fig. 1; feeding regime × age: $F_{10,611} = 16.83$, $P < 0.001$). In addition, we detected a significant interaction between feeding regime and incubation temperature on duckling food consumption (food regime × incubation temperature: $F_{1,163} = 3.65$, $P = 0.028$). Whereas ducklings fed the restricted diet ate similar amounts regardless of their prior incubation temperature, ducklings fed the ad lib. diet from the low incubation temperature ate 8%–24% less food than ducklings fed ad lib. from the medium and high incubation temperatures. The influence of incubation temperature on food consumption was consistent across ages (age × incubation temperature: $P = 0.93$; age × incubation temperature × feeding regime: $P = 0.79$).

Ducklings from all treatment groups increased in mass with age (table 3; fig. 2); however, the degree to which mass increased depended on both incubation temperature and feeding regime (fig. 2; incubation temperature × food regime × age: $F_{24,1,008} = 2.11$, $P = 0.001$). Growth was lower in ducklings with restricted access to resources compared with ducklings fed ad lib. Contrary to our predictions, however, all ducklings fed a restricted diet exhibited similar increases in mass over time, regardless of their prior incubation temperature. In contrast, ducklings from the low incubation temperature fed an ad lib. diet had ~7% lower body mass by 6 d posthatch than ducklings on the ad lib. diet from the medium and high incubation temperatures, and this effect became more pronounced with age (fig. 2).

Regardless of feeding regime, ducklings from the highest incubation temperature were in better body condition than ducklings from the lower two incubation temperatures (table 3; fig. 3; incubation temperature: $F_{2,85} = 6.22$, $P = 0.003$). As expected, ducklings on the ad lib. diet were in better body condition than ducklings on the restricted diet, and this pattern also became more pronounced with duckling age (feeding regime × age: $F_{4,340} = 5.00$, $P < 0.001$). Post hoc investigation of individual ANOVAs revealed that incubation temperature was significant at every age. There was not, however, an interactive effect of incubation temperature and feeding regime on duckling body condition (incubation temperature × feeding regime:

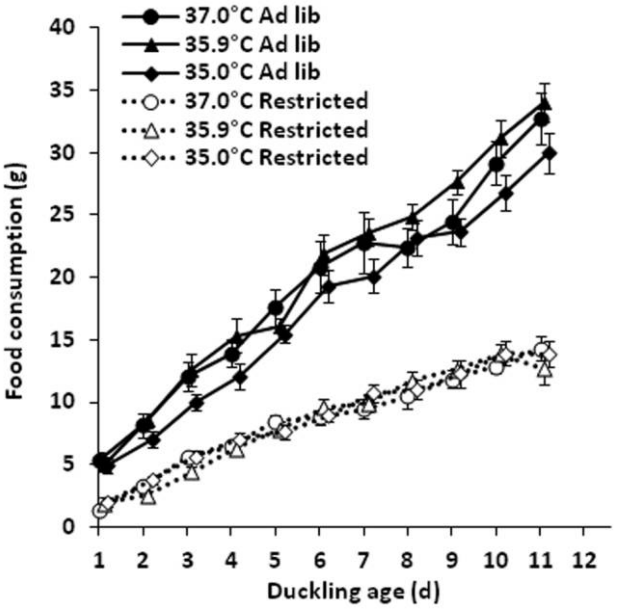

Figure 1. Food consumption (means ± 1 SE) of ducklings from 1 to 11 d posthatch that were exposed twice to 10°C thermal challenges. Ducklings were reared on an ad lib. or restricted diet and hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C).

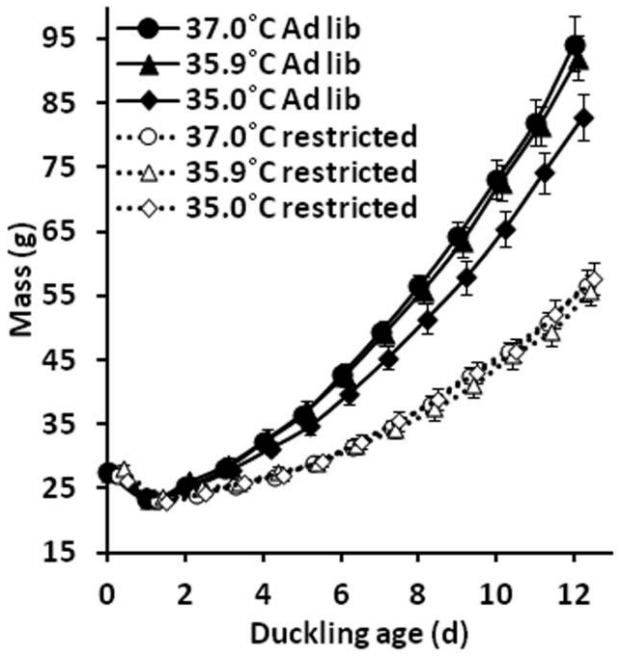

Figure 2. Body mass (means ± 1 SE) of ducklings from 1 to 12 d posthatch that were exposed twice to 10°C thermal challenges. Ducklings were reared on an ad lib. or restricted diet and hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C).

$F_{2,85} = 0.90$, $P = 0.409$; incubation temperature × feeding regime × age: $F_{8,336} = 1.22$, $P = 0.307$).

Ducklings from the high incubation temperature maintained their body temperature better at 4 d posthatch than at 1 d posthatch, while ducklings from the medium and low incubation temperature did not improve thermoregulatory ability with age (tables 3, 4; fig. 4; age × incubation temperature: $F_{2,86} = 7.48$, $P = 0.001$). Contrary to our prediction, the effect of incubation temperature on duckling thermoregulation was consistent across feeding regimes (incubation temperature × feeding regime: $P = 0.401$; age × incubation temperature × food regime: $P = 0.824$). Although not statistically significant, there was some evidence that ducklings fed the restricted diet experienced smaller decreases in body temperature than ducklings fed ad lib. (feeding regime: $F_{2,86} = 3.00$, $P = 0.087$; age × feeding regime: $P = 0.798$). Duckling mass also influenced the percent change in body temperature during a thermal challenge ($F_{1,86} = 10.74$, $P = 0.002$), with heavier ducklings experiencing smaller drops in body temperature than lighter ducklings.

Consistent with our predictions, incubation temperature significantly affected how well ducklings improved at thermoregulating from 1 to 4 d posthatch (fig. 5; incubation temperature: $F_{2,86} = 5.19$, $P = 0.008$). Ducklings from the high incubation temperature showed substantial improvement, whereas ducklings from the medium and low incubation temperature did not improve significantly with time. In contrast, there was no effect of feeding regime on the improvement in duckling thermoregulation with age (feeding regime: $F_{1,86} = 0.11$, $P = 0.745$), and feeding regime did not interact with incubation temperature to influence ducklings' thermoregulatory maturation (incubation temperature × feeding regime: $F_{2,86} = 0.19$, $P = 0.829$). Duckling mass at 1 d posthatch had a marginally positive influence on thermoregulatory ability ($F_{1,86} = 3.19$, $P = 0.078$).

Discussion

Most studies evaluating the importance of incubation temperature to reproductive success in birds have focused on nest predation risks, length of the incubation period, hatching success, and body size of the hatchling (Webb 1987; Zicus et al. 1995; Tombre and Erikstad 1996; Nuechterlein and Buitron 2002; Reid et al. 2002; Hepp et al. 2006). Fewer studies have examined effects on physiology and performance of the young after hatching (Nilsson et al. 2008; Ardia et al. 2010; DuRant et al. 2010, 2012*a*, 2012*b*; Hopkins et al. 2011; Nord and Nilsson 2011), whether these effects persist (DuRant et al. 2010, 2012*a*; Hopkins et al. 2011), and how effects manifest under different posthatching environments (Nilsson et al. 2008; Ardia et al. 2010). Taken together, these studies clearly demonstrate that temperatures experienced during incubation have tremendous implications for expression of phenotypes that could influence fitness of both parents and their offspring. However, the question remains whether these effects are reversible or can be tem-

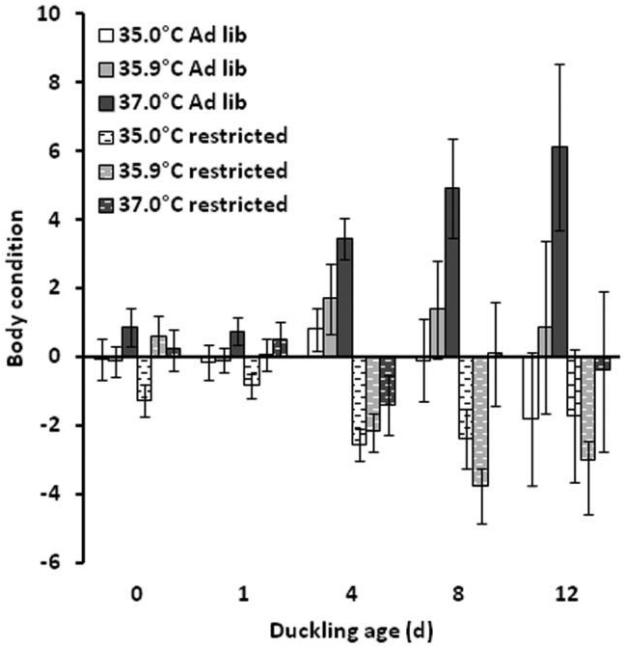

Figure 3. Body condition (residuals of mass vs. tarsus; means ± 1 SE) of ducklings at 0, 1, 4, 8, and 12 d posthatch that were exposed twice to 10°C thermal challenges. Body condition is presented here as the residuals of mass versus tarsus. Ducklings were reared on an ad lib. or restricted diet and hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C).

Table 4: Body temperature (mean ± 1 SE) of ducklings held at thermoneutral (36°C) and outside of thermoneutral (10°C) temperatures

	Day 1		Day 4	
Treatment	36°C	10°C	36°C	10°C
Ad lib.:				
37.0°C	38.9 ± .3	34.8 ± .7	39.6 ± .2	37.9 ± .3
35.9°C	39.3 ± .2	37.1 ± .2	40.0 ± .2	38.3 ± .2
35.0°C	39.0 ± .2	35.7 ± .9	39.9 ± .2	37.3 ± .8
Restricted:				
37.0°C	39.0 ± .3	36.1 ± .5	39.7 ± .2	38.4 ± .4
35.9°C	39.2 ± .2	37.0 ± .4	39.4 ± .2	36.9 ± .6
35.0°C	39.1 ± .2	37.0 ± .3	39.6 ± .2	37.1 ± .8

Note. Ducklings hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, and 37.0°C) and after hatching were reared on an either ad lib. or restricted feeding regime.

pered by the posthatching resource environment. To our knowledge, this is the first study to determine how the carryover effects of incubation temperature vary under different resource conditions after hatching.

In this study, we found that incubation temperature influenced growth, body condition, and thermoregulation of ducklings, with the poorest performance occurring in ducklings that developed at the lowest incubation temperature. Moreover, the prehatch developmental environment was more important to thermoregulatory performance than the posthatch environment (figs. 4, 5), whereas both pre- and posthatch conditions influenced growth (figs. 2, 3). These findings suggest that for precocial birds, the investments made by parents to maintain optimal incubation temperatures are crucial because offspring may not be able to overcome the consequences of being incubated at cooler temperatures. Our findings support previous research demonstrating that incubation temperature plays a pivotal role in determining avian offspring phenotype (Hepp et al. 2006; DuRant et al. 2010, 2012*a*, 2012*b*; Hopkins et al. 2011; Nord and Nilsson 2011), but our work extends these findings to suggest that the incubation environment plays a greater role in shaping some phenotypic traits than certain environmental conditions experienced posthatch.

After ducklings hatched, they were randomly placed on a food-restricted or ad lib. diet. When food was restricted, ducklings from all incubation temperatures consumed similar amounts of food (fig. 1). In contrast, when food was provided ad lib., ducklings from the low incubation temperature tended to consume less food than ducklings from the higher incubation temperatures. Growth patterns were consistent with food consumption patterns; growth was similar among incubation temperatures for ducklings fed the restricted diet, but under ad lib. feeding conditions, low incubation temperature ducklings had slower growth than higher incubation temperature ducklings (fig. 2). The effect of incubation temperature on growth of ducklings fed the ad lib. diet is consistent with previous studies that demonstrated that effects of incubation temperature on growth persist until at least 20 d posthatch (DuRant et al. 2010; DuRant et al. 2012*a*). Presumably, ducklings from the restricted group all ate similar quantities of food regardless of incubation temperature because they never were allowed to feed to satiation and simply consumed as much food as possible when food was present. However, it is unclear why ducklings from the low incubation temperature fed ad lib. consumed less food than ducklings fed ad lib. from the two higher temperatures, but it may have to do with differences in motivation to eat. Recent work in our lab indicates that ducklings from the lowest incubation temperature have lower T_3 hormone concentrations (S. E. DuRant, unpublished data), a hormone important for both growth and feeding in birds (McNabb and King 1993;

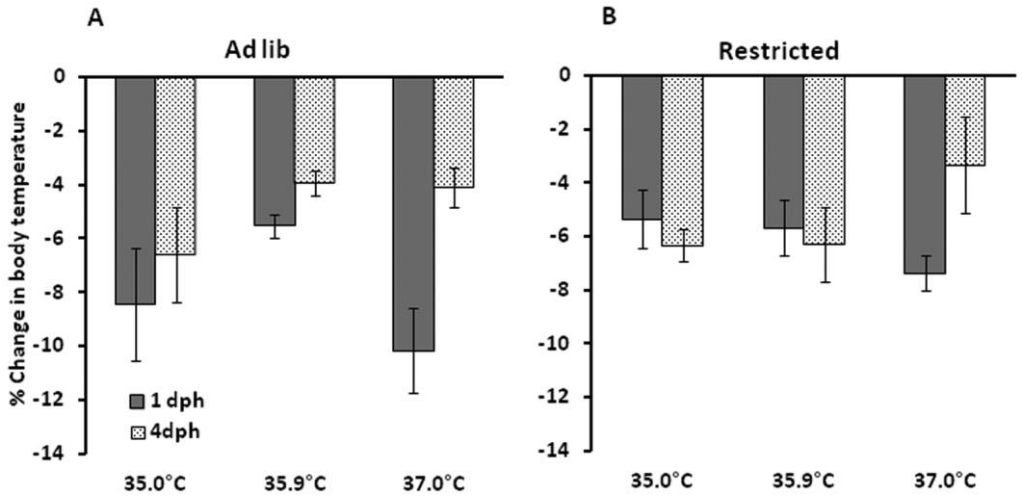

Figure 4. Percent change (means ± 1 SE) in 1 and 4 d posthatch (dph) duckling body temperature from before to after exposure to a 1-h thermal challenge at 10°C. Ducklings were reared on an ad lib. diet (*A*) or a restricted diet (*B*) and hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C).

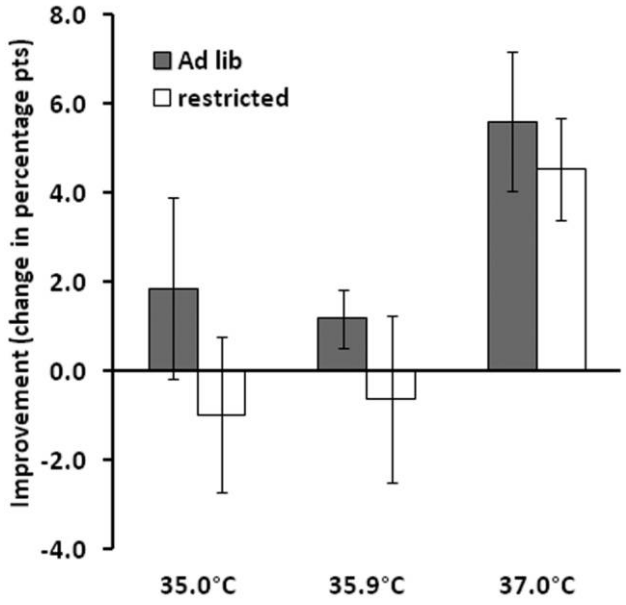

Figure 5. Improvement in duckling ability to maintain its body temperature (means ± 1 SE) from 1 to 4 d posthatch when exposed to a 1-h thermal challenge at 10°C. Ducklings were reared on an ad lib. diet (gray bars) or a restricted diet (white bars) and hatched from eggs incubated at one of three temperatures (35.0°, 35.9°, or 37.0°C).

McNabb et al. 1998). Alternatively, reduced feeding in ducklings from the low incubation temperature fed ad lib. could be attributable to their smaller size at hatching, which may lower their food requirements. However, this effect was not evident in birds fed the restricted diet. Regardless of the mechanism, the consequences of reduced food consumption are clearly evident in duckling growth profiles.

Contrary to the dual influence of pre- and posthatch environments on duckling growth, only the prehatch environment influenced duckling thermoregulation (figs. 4, 5). Ducklings that hatched from eggs incubated at the highest incubation temperature showed significantly more improvement in maintaining their body temperature from 1 to 4 d posthatch than ducklings in the lower incubation temperatures, regardless of posthatch feeding conditions. Thus, ducklings from the highest incubation temperature may transition to endothermy more quickly than ducklings from the other incubation temperatures. Interestingly, similar to many other phenotypic characteristics we have examined (DuRant et al. 2010, 2012*a*, 2012*b*, 2013*b*), incubation temperature does not affect thermoregulatory ability in a linear fashion (figs. 4, 5). Importantly, there was no effect of feeding conditions on duckling thermoregulation, even though body mass between ad lib. and restricted ducklings differed by ~8% at 4 d posthatch. This finding suggests that the effect of incubation temperature on thermoregulation may be controlled by physiological factors independent of factors influenced by body mass. For example, developmental delays or differences in muscle maturity, thyroid hormone production or action, or ability to detect and thus respond to changes in ambient temperature could all underlie our observations (Visser and Ricklefs 1993, 1995; McNabb et al. 1998; Visser 1998; DuRant et al. 2010, 2012*b*). Regardless of how incubation temperature influenced thermoregulatory ability of ducklings, the differences in thermoregulatory ability among treatments could translate into differences in survival. Early thermoregulation is essential to the early survival of highly precocial avian offspring. Indeed, in some species of waterfowl, hypothermia can account for 8%–25% of hatchling mortality occurring within the first days of hatching (Talent et al. 1983; Mauser et al. 1994; Korschgen et al. 1996). Alternatively, the decreases in body temperature experienced during the thermal trial could be interpreted as adaptive, because resilience to decreases in body temperature is thought to allow young chicks to transition to homeothermy without constant reliance on brooding or huddling while still allowing the chick to remain active at comparatively low energetic costs (Visser 1998; see also Angilletta 2010). However, previous work in our lab indicated that ducklings from the lowest incubation temperature expend more energy when thermoregulating than do ducklings from the higher incubation temperatures, suggesting that these thermoregulatory deficiencies come at a cost that is likely detrimental (DuRant et al. 2012*b*).

In addition to affecting posthatching characteristics of ducklings, incubation temperature also influenced duckling size at hatching (tables 1, 2), consistent with the findings of previous studies (Hepp et al. 2006; DuRant 2012*b*; but see DuRant et al. 2010, 2012*a*). Ducklings from the highest incubation temperature had higher body mass at hatching than ducklings from the lower incubation temperatures, a result at least partially attributed to shorter incubation duration and lower total energy expenditure during incubation at higher temperatures (DuRant et al. 2011). Heavier body size can have fitness advantages. For instance, heavier mallard ducklings have improved survival rates to 30 d posthatch (Amnudson and Arnold 2011), and heavier ducklings have greater thermoregulatory capabilities (Rhymer 1988). Interestingly, ducklings from the high incubation temperature hatch with smaller structural size (tarsus length; Hepp et al. 2006; this study) than ducklings from the lower temperatures. Although embryonic development is slower at the lower temperatures (Booth 1987; Olson et al. 2006; DuRant et al. 2011), ducklings experiencing longer developmental periods grow structurally larger but use more energy to do so and thus hatch with lower body mass and condition.

The importance of incubation temperature to avian phenotypic expression has been substantiated by a growing body of literature. Our study not only corroborates those findings but also further underscores the importance of the prehatch environment for the expression of some phenotypic traits early in ontogeny by directly comparing its importance to the influence of posthatch resource conditions. Our findings suggest that precocial birds may incur fitness benefits by investing energy in optimal incubation environments, since their young hatch with phenotypes that are more conducive to early survival. However, maintenance of incubation conditions comes at costs to incubating parents because it detracts from self-

maintenance (e.g., decreased body mass, immune responses, and survival), creating potential trade-offs between current and future reproductive success (Reid et al. 2000*a*, 2000*b*; Hansenn et al. 2005; de Heij et al. 2006; DuRant et al. 2013*a*). Further studies are needed to compare the relative importance of incubation temperature and posthatch conditions to phenotypic expression in altricial and precocial species. Since altricial young are still rapidly developing and maturing tissues after hatching, their phenotype may be more plastic at hatching; thus, parents may still be able to compensate for suboptimal incubation conditions by providing a better nest environment after hatching. Even if offspring that experience suboptimal temperatures during incubation can offset the phenotypic effects of developmental conditions by consuming more resources posthatch, multiple lines of evidence demonstrate that there are costs to such compensatory mechanisms (Metcalfe and Monaghan 2001; Monaghan 2008). For instance, costs associated with compensatory growth have been documented in a range of taxa and can include higher predation risk, lower starvation resistance and locomotor performance, and shorter life span (reviewed in Metcalfe and Monaghan 2001). Taken together, our current findings in conjunction with previous research on temperature-induced avian phenotypes (DuRant et al. 2013*b*) highlight the importance of incubation temperatures to avian ecology and life history and also underscore the importance of conserving nesting habitat and minimizing disturbance experienced by incubating birds, given the repercussions that suboptimal temperatures have on offspring phenotype.

Acknowledgments

We thank Thera Lombardi, Stephanie Chin, David Drewett, Ashley Love, Mark Hepner, Jake McPherson, Maureen McClintock, Assley Garner, and Bobby Kennamer for their help in the field or laboratory. Primary funding for this research was supported by National Science Foundation (NSF) grants IOB-0615361 to G.R.H. and W.A.H. and IOS-1048529 to L. Michael Romero and an NSF Doctoral Dissertation Improvement Grant (DEB-1110386) to S.E.D. and W.A.H. Additional support was provided by grants from Sigma Xi, the Society of Integrative and Comparative Biology, and the Virginia Tech Graduate Research and Development program awarded to S.E.D. All methods were university approved, and we obtained all necessary collection permits: Institutional Animal Care and Use Committee approved proposal 11-056-FIW; collection permits: South Carolina Department of Natural Resources G-08-07; US Fish and Wildlife Service MB748024-0. This material is based on work supported by the Department of Energy under award DE-FC09-07SR22506 to the University of Georgia Research Foundation.

Literature Cited

Amundson C.L. and T.W. Arnold. 2011. The role of predator removal, density-dependence, and environmental factors on mallard duckling survival in North Dakota. J Wildl Manag 75:1330–1339.

Angilletta M.J., Jr., B.S. Cooper, M.S. Schuler, and J.G. Boyles. 2010. The evolution of thermal physiology in endotherms. Front Biosci E2:861–881.

Ar A. and Y. Sidis. 2002. Nest microclimate during incubation. Pp. 143–160 in D.C. Deeming, ed. Avian incubation: behaviour, environment, and evolution. Oxford University Press, New York.

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

Bellrose F.C. and D.J. Holm. 1994. Ecology and management of the wood duck. Stackpole, Mechanicsburg, PA.

Biard C., D. Gil, F. Karadas, N. Saino, C.N. Spottiswoode, P.F. Surai, and A.P. Møller. 2009. Maternal effects mediated by antioxidants and the evolution of carotenoid-based signals in birds. Am Nat 174:696–708.

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

Caldwell P.J. 1972. Development of thermoregulation in mallard ducklings. Condor 75:113–114.

Congdon J.D., R.D. Nagle, A.E. Dunham, C.W. Beck, O.M. Kinney, and S.R. Yeomans. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentine*): an evaluation of the "bigger is better" hypothesis. Oecologia 121:224–235.

Cox R.R., Jr., M.A. Hanson, C.C. Roy, N.H. Euliss Jr., D.H. Johnson, and M.G. Butler. 1998. Mallard duckling growth and survival in relation to aquatic invertebrates. J Wildl Manag 62:124–133.

Cucco M. and G. Malacarne. 2009. Effect of food availability on nestling growth and fledging success in manipulated pallid swift broods. J Zool 240:141–151.

Deeming D.C. and M.W.J. Ferguson. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. Pp. 147–171 in D.C. Deeming and M.W.J. Ferguson, eds. Egg incubation: its effects on embryonic development in birds and reptile. Cambridge University Press, New York.

de Heij M.E., P.J. van den Hout, and J.M. Tinbergen. 2006. Fitness cost of incubation in great tits (*Parus major*) is related to clutch size. Proc R Soc B 273:2353–2361.

Dunn P.O. and S.J. Hannon. 1992. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. Auk 109:488–499.

DuRant S.E., G.R. Hepp, I.T. Moore, B.C. Hopkins, and W.A. Hopkins. 2010. Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. J Exp Biol 213:44–51.

DuRant S.E., W.A. Hopkins, D.M. Hawley, and G.R. Hepp. 2012*a*. Incubation temperature affects multiple measures of immunocompetence in wood duck ducklings. Biol Letters 8: 108–111.

DuRant S.E., W.A. Hopkins, and G.R. Hepp. 2011. Energy ex-

penditure of developing wood duck (*Aix sponsa*) embryos is related to incubation temperature. Physiol Biochem Zool 84: 451–457.

DuRant S.E., W.A. Hopkins, G.R. Hepp, and L.M. Romero. 2013*a*. Energetic constraints and parental care: is corticosterone an important mediator of incubation behavior in a precocial bird? Horm Behav 63:385–391.

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

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

Einum S. and I.A. Fleming. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54:628–639.

Eising C.M., W. Müller, and T.G.G. Groothuis. 2006. Avian mothers create different phenotypes by hormone deposition in their eggs. Biol Lett 2:20–22.

Groothuis T.G.G., W. Müller, N. von Engelhardt, C. Carere, and C. Eising. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neurosci Biobehav Rev 29:329–352.

Groothuis T.G.G. and H. Schwabl. 2008. Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? Philos Trans R Soc B 363:1647–1661.

Gunnarsson G., J. Elberg, K. Sjoberg, H. Poysa, and P. Nummi. 2004. Why are there so many empty lakes? food limits survival of mallard ducklings. Can J Zool 82:1698–1703.

Hanssen S.A., D. Hasselquist, I. Folstad, and K.E. Erikstad. 2005. Cost of reproduction in long-lived birds: incubation effort reduces immune function and future reproduction. Proc R Soc B 272:1039–1046.

Hepp G.R. and F.C. Bellrose. 1995. The Birds of North America No. 169. American Ornithologists' Union and Academy of Natural Sciences of Philadelphia, Washington, DC.

Hepp G.R. and R.A. Kennamer. 2012. Warm is better: incubation temperature influences apparent survival and recruitment of wood ducks (*Aix sponsa*). PLoS ONE 7:e47777, doi: 10.1371/journal.pone.0047777.

Hepp G.R., R.A. Kennamer, and M.H. Johnson. 2006. Maternal effects in wood ducks: incubation temperature influence incubation period and neonate phenotype. Funct Ecol 20:307–314.

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

Kitaysky A.S., E.V. Kitaiskaia, J.F. Piatt, and J.C. Wingfield. 2006. A mechanistic link between chick diet and decline in seabirds? Proc R Soc B 273:445–450.

Korschgen C.E., K.P. Kenow, W.L. Green, D.H. Johnson, M.D. Samuel, and L. Sileo. 1996. Survival of radio-marked canvasback ducklings in northwestern Minnesota. J Wildl Manag 60:120–132.

Koskimies J. and L. Lahti 1964. Cold-hardiness of the newly hatched young in relation to ecology and distribution in ten species of European ducks. Auk 81:281–307.

Lepage D., G. Gauthier, and A. Reed. 1998. Seasonal variation in growth of greater snow goose goslings: the role of food supply. Oecologia 114:226–235.

Manlove C.A. and G.R. Hepp. 2000. Patterns of nest attendance in female wood ducks. Condor 102:286–291.

Martin T.E., S.K. Auer, R.D. Bassar, A.M. Niklison, and P. Lloyd. 2007. Geographic variation in avian incubation periods and parental influences on embryonic temperature. Evolution 61: 2558–2569.

Mauser D.M., R.L. Jarvis, and D.S. Gilmer. 1994. Survival of radiomarked mallard ducklings in northeastern California. J Wildl Manag 58:82–87.

McNabb F.M.A. and D.B. King. 1993. Thyroid hormone effects on growth, development and metabolism. Pp. 393–417 in M.P. Schreibman, C.G. Scanes, and P.K.T. Pang, eds. The endocrinology of growth, development, and metabolism of vertebrates. Academic Press, New York.

McNabb F.M.A., C.G. Scanes, and M. Zeman. 1998. Endocrine control of development. Pp. 174–202 in J.M. Starcks and R.E. Rickefs, eds. Avian growth and development. Oxford University Press, New York.

Metcalfe N.B. and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–260.

Monaghan P. 2008. Early growth conditions, phenotypic development, and environmental change. Philos Trans R Soc B 363:1635–1645.

Neuchterlein G.L. and D. Buitron. 2002. Nocturnal egg neglect and prolonged incubation in the red-necked grebe. Waterbirds 25:485–491.

Nilsson J.F., M. Stjemman, and J. Nilsson. 2008. Experimental reduction of incubation temperature affects both nestling and adult blue tits *Cyanistes caeruleus*. J Avian Biol 39:553–559.

Nord A. and J.A. Nilsson. 2011. Incubation temperature affects growth and energy metabolism in blue tit nestlings. Am Nat 5:639–651.

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

Reid J.M., E.M. Bignal, S. Bignal, D.I. McCracken, and P. Monaghan. 2006. Spatial variation in demography and population growth rate: the importance of natal location. J Anim Ecol 75:1201–1211.

Reid J.M., P. Monaghan, and R.G. Nager. 2002. Incubation and the costs of reproduction. Pp. 314–325 in D.C. Deeming, ed. Avian incubation: behavior, environment, and evolution. Oxford University Press, New York.

Reid J.M., P. Monaghan, and G.D. Ruxton. 2000*a*. The consequences of clutch size for incubation conditions and hatching success in starlings. Funct Ecol 14:560–565.

———. 2000*b*. Resource allocation between reproductive

phases: the importance of thermal conditions in determining the cost of incubation. Proc R Soc B 267:37–41.

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

Romano M.D., J.F. Piatt, and D.D. Roby. 2006. Testing the junk-food hypothesis on marine birds: effects of prey type on growth and development. Waterbirds 29:407–414.

Saino N., R. Ferrari, M. Romano, R. Martinelli, and A.P. Møller. 2003. Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. Proc R Soc B 270:2485–2489.

Schew W.A. 1995. The evolutionary significance of developmental plasticity in growing birds. PhD diss. University of Pennsylvania, Philadelphia.

Schew W.A., F.M.A. McNabb, and C.G. Scanes. 1996. Comparison of the ontogenesis of thyroid hormones, growth hormone, and insulin-like growth factor-I in ad libitum and food-restricted (altricial) European starlings and (precocial) Japanese quail. Gen Comp Endocrinol 101:304–316.

Talent L.G., R.L. Jarvis, and G.L. Krapu. 1983. Survival of mallard broods in south-central North Dakota. Condor 85:74–78.

Tombre I.M. and K.E. Erikstad. 1996. An experimental study of incubation effort in high-Arctic barnacle geese. J Anim Ecol 65:325–331.

Tremblay I., D. Thomas, J. Blondel, P. Perret, and M.M. Lambrechts. 2005. The effect of habitat quality on foraging patterns, provisioning rate, and nestling growth in Corsican blue tits *Parus caeruleus*. Ibis 147:17–24.

Uttley J.D., P. Walton, P. Monaghan, and G. Austin. 1994. The effects of food abundance on breeding performance and adult time budgets of guillemots *Uria allge*. Ibis 136:205–213.

Visser G.H. 1998. Development of temperature regulation. Pp. 117–156 in J.M. Starck and R.E. Ricklefs, eds. Avian growth and development: evolution within the altricial-precocial spectrum. Oxford University Press, New York.

Visser G.H. and R.E. Ricklefs. 1993. Temperature regulation in neonates of shorebirds. Auk 110:445–457.

———. 1995. Relationship between body composition and homeothermy in neonates of precocial and semiprecocial birds. Auk 112:192–200.

Walls J.G., G.R. Hepp, and L.G. Eckhardt. 2011. Effects of incubation delay on viability and microbial growth of wood duck (*Aix sponsa*) eggs. Auk 128:663–670.

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

White F.N. and J.L. Kinney. 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. Biol Rev 68:35–59.

Zicus M.C., S.K. Hennes, and M.R. Riggs. 1995. Common goldeneye nest attendance patterns. Condor 97:461–472.

Zubair A.K. and S. Leeson. 1994. Effect of early restriction and realimentation on heat production and changes in sizes of digestive organs of male broilers. Poult Sci 73:529–538.