CHAPTER 14

Influence of incubation temperature on offspring phenotype and fitness in birds

G.R. Hepp, S.E. DuRant, and W.A. Hopkins

14.1 Introduction

Incubation is an important reproductive cost in birds, particularly in species that use contact incubation to warm eggs (Visser and Lessells 2001; Tinbergen and Williams 2002). Costs manifest as constraints on the allocation of energy, nutrients, and time to competing processes such as self-maintenance. Metabolic costs to parents increase during incubation, especially at ambient temperatures below thermoneutrality and in species where only one parent incubates (Williams 1996; Tinbergen and Williams 2002). However, incubating parents must spend time on the nest providing heat to eggs, so less time is available for foraging to meet their increased energy needs. Nutrient reserves can help to satisfy these energy demands, but the ability to use endogenous energy declines with body mass of species and individuals (Afton and Paulus 1992). In some species, feeding of incubating females by their male partner also can help females to balance daily energy costs (Lyon and Montgomerie 1985; Galván and Sanz 2011). However, it is clear that incubation costs are important and can influence both current and future reproductive success (Visser and Lessells 2001; Reid et al. 2000a, 2002b; Hanssen et al. 2005; Hepp and Kennamer 2012; DuRant et al. 2013a) and impact the evolution of life histories (Monaghan and Nager 1997; Martin 2008). Incubating individuals, therefore, are challenged to balance the competing demands of self-maintenance with the thermal requirements of developing embryos.

Low incubation temperature slows development leading to longer incubation periods, reduced neonate ‘quality’, and increased risk of nest predation (Tombre and Erikstad 1996; Hepp et al. 2006; Martin et al. 2007, 2013a; DuRant et al. 2013a). Even small differences in incubation temperature can have significant impacts (reviewed in DuRant et al. 2013a). Therefore, it is important for incubating parents to provide a thermal environment that allows for normal embryo development although incubation temperatures can fall below this level especially during incubation recesses (Martin and Schwabl 2008).

Energy constraints and predation risk are two of the prominent factors that influence nest attentiveness and incubation temperatures. For example, females of several passerine species spent less time on the nest and were unable to maintain optimum incubation temperatures after incubation costs were experimentally increased (Ardia et al. 2010; Nord et al. 2010; Nord and Nilsson 2012). However, when incubation costs were reduced by heating nests, females increased nest attentiveness and incubation temperatures (Bryan and Bryant 1999; Ardia et al. 2009). These studies show that energy constraints during incubation are important and influence trade-offs between self-maintenance and embryo development. Predation risk can also influence parental investment decisions, independent of energy constraints. High risks of predation, for example, cause parents to reduce nest attentiveness resulting in lower incubation temperatures and longer incubation periods (Conway and Martin 2000b; Martin 2002; Martin et al. 2007).

Variation in parental quality certainly influences how parents respond to reproductive costs (Cam et al. 1998; Sanz-Aguilar et al. 2008), including those of...
incubation. For example, as incubation costs increased with increasing clutch size in common eiders (Somateria mollissima), females in high body condition invested more in incubation and subsequent brood care than did females in low body condition (Hanssen et al. 2003). Ardia and Clotfelter (2007) altered the energy constraints of incubating female tree swallows (Tachycineta bicolor) by clipping their primary feathers forcing them to work harder during foraging. They found that early-nesting females in high body condition were affected less by the feather-clipping manipulation and spent more time incubating and produced nestlings in higher body condition than late-nesting females in low body condition. In wood ducks (Aix sponsa), females in low body condition nested later in the season than females in higher body condition (Hepp and Kennamer 1993). Late-nesting wood ducks reduced incubation costs by spending less time on the nest, but incubation temperatures were similar to those of early-nesting females because ambient temperatures were higher late in the season (Hepp and Kennamer 2011). These experimental studies make it clear that incubation costs are not homogeneous but depend on parental body condition.

### 14.3 Factors influencing incubation temperature

Parents use a variety of methods to achieve and maintain optimal egg temperatures during incubation (Ardia and Sidis 2002; Deeming 2002e). Incubation behaviour is a key factor affecting incubation temperature, and variation in behaviours underlying the number and length of recesses and overall incubation constancy are influenced by factors such as ambient temperature, precipitation, predation risk, egg size, and body condition (Conway and Martin 2000b; Deeming 2002e; Tombre et al. 2012). Behavioural plasticity allows parents to modify their response to current environmental conditions. Female tree swallows responded to the experimental heating and cooling of nests by increasing and decreasing, respectively, time spent incubating (Ardia et al. 2009, 2010). Under natural conditions, female tree swallows modulate the amount of time they stay off the nest according to precipitation and temperature patterns (Coe et al. 2014). During cold and dry conditions, females stayed off their nests longer than under warm and dry conditions but this pattern was reversed under periods of heavy rainfall; females tended to take shorter off-bouts when it was rainy and cold compared to longer off-bouts during warmer rain events. As expected, variation in female tree swallow behaviour was associated with differences in overall incubation temperature, such that females that took shorter, more frequent off-bouts maintained higher incubation temperatures than those that opted for longer, less frequent off-bouts (Coe et al. 2014). Similarly, female wood ducks successfully maintained optimal egg temperatures by adjusting daily incubation behaviours in response to variation in both ambient temperature and clutch cooling rates (McClintock et al. 2014). Martin et al. (2007) examined 41 passerine species on three continents and showed that reduced levels of parental nest attentiveness resulted in lower egg temperatures and longer incubation periods. Together, these studies clearly demonstrate that parental incubation behaviour is a key determinant of incubation temperature, but understanding the proximate causes of variable parental incubation behaviour remains an area ripe for future study.

Size, structure, and composition of nests influence their thermal properties and affect the energetic costs of incubation (Smith et al. 1974) as well as the parent’s ability to provide an appropriate microclimate for the eggs (Skowron and Kern 1980; Lombardo et al. 1995; Hansell and Deeming 2002; Hilton et al. 2004; Heenan 2013; Mainwaring et al. 2014b; Chapter 4). Yellow warblers (Dendroica petechia) nesting at Churchill, Manitoba, for example, built larger, thicker nests that cooled more slowly than nests from a more southerly location in Ontario (Rohwer and Law 2010). Similarly, blue tits (Cyanistes caeruleus) and great tits (Parus major) consistently built better-insulated nests at higher latitudes in the UK where ambient temperatures were cooler (Mainwaring et al. 2012). Many bird species improve the insulatory quality of nests by adding feathers (see Chapter 3). McClintock et al. (2014) manipulated the amount of down insulation in wood duck nests and showed that nests with reduced down (0.5 g) cooled 20% faster than nests with normal amounts of down (4.0 g). Tree swallow eggs also cooled faster in nests without feathers than in nests with them (Windsor et al. 2013). Furthermore, tree swallow nests in which feathers were experimentally removed had longer incubation periods and produced fewer fledglings than nests with feathers (Lombardo et al. 1995).

Nest location affects nest microclimate by influencing exposure of nests to environmental conditions (Hartman and Oring 2003; Ardia et al. 2006b). Therefore, selection of nest sites is important because of the potential impact that nest microclimate has on reproductive success (Lloyd and Martin 2004; Salaberria et al. 2014). Tree pipits (Anthus trivialis), for example, preferred to build nests with an eastward orientation...
that protected eggs from direct sunlight in the middle of the day which improved hatching success of these nests compared to nests with different orientations (Burton 2006). Similarly, vegetative cover at nest sites selected by vesper sparrows (Poecetes gramineus) and horned larks (Eremophila alpestris) protected nests from solar radiation and resulted in a more favourable nest microclimate (Nelson and Martin 1999).

14.4 Incubation temperature and avian phenotypes

Effect of incubation temperature on hatching phenotypes has been widely studied in reptiles (e.g. Brown and Shine 2004; Deeming 2004b; Booth 2006; Mitchell et al. 2013; Du and Shine 2015) and domestic poultry (Deeming and Ferguson 1991b), but only recently has it been examined in wild birds (DuRant et al. 2013a).

Poultry scientists have been interested in incubation temperature because of its potential economic importance related to hatching success and early growth and performance of chicks (Lourens et al. 2005; Hulet et al. 2007). In domestic fowl (Gallus gallus), for example, chronic low incubation temperature (35°C) lengthens the incubation period, and reduces hatching success and the ability of neonates to thermoregulate (Black and Burggren 2004a, 2004b). Changes in temperature during just a portion of the incubation period also can impact embryo development. When temperatures were altered in late incubation (days 10–18), chicken eggs incubated at low temperatures (33.3–36.7°C) had longer incubation periods and produced chicks with reduced body mass at hatching and at 7 days of age compared to eggs incubated at mid (37.8–38.2°C) and high (38.9–40.0°C) temperatures (Ipek et al. 2014). Incubation temperatures early in the incubation period also influence embryo development. Eggs incubated at low temperatures (36.6°C) early in incubation (days 0–10) and then incubated at normal temperatures (37.8°C) for the remainder of incubation had reduced hatching success and chicks were smaller at 1, 3, and 6 weeks of age compared to those incubated constantly at 37.8°C (Joseph et al. 2006). Studies of incubation temperature in poultry may not be rooted in an ecological framework, but nevertheless they provide important insights into the potential importance of incubation temperature in wild birds.

Parental effects such as maternal transfer of hormones to eggs can help to prepare neonates for environmental conditions they will experience after hatching (Mousseau and Fox 1998b; Love and Williams 2008a; Bentz et al. 2013) but this is not always the case (Monaghan 2008). Growth and development of nestling great tits, for example, were not enhanced when food resources were equivalent pre- and post-hatch (Giordano et al. 2014). Similarly, phenotypic changes to wild avian neonates caused by sub-optimal incubation temperatures do not appear to be adaptive (DuRant et al. 2013a). Recent studies, especially of wood ducks and tree swallows, have examined effects of incubation temperature on a suite of characters that are important to breeding success. Together, these studies suggest that sub-optimal temperatures produce phenotypes that would perform unfavourably under most environmental conditions. Here, we summarize those findings and discuss their potential effects on fitness.

14.4.1 Body size, body composition, and growth

Several studies have artificially incubated eggs at ecologically relevant temperatures, and measured effects of temperature on the mass, structural size, composition, and subsequent growth of recently hatched chicks. In a megapode, the Australian brush-turkey (Alectura lathami), eggs were incubated at 32°C, 34°C, and 36°C, and chicks from higher temperatures had shorter incubation periods and tended to be structurally smaller, have more residual yolk and reduced yolk-free body mass compared with chicks incubated at lower temperatures (Eiby and Booth 2009). Hepp and Kennamer (2012) incubated wood duck eggs at 35.0°C, 35.9°C, and 37.3°C, and found that body mass of recently hatched ducklings declined and tarsus length increased with lower incubation temperatures. Furthermore, ducklings incubated at the lowest temperature used more lipids during a longer incubation period and hatched with 20% fewer lipids than ducklings incubated at the higher temperatures (Hepp and Kennamer 2012). In other studies, the effect of incubation temperature (35.0°C, 35.9°C, and 37.0°C) on wood duck hatching mass varied somewhat. Body mass and condition of hatchlings were greatest for hatchlings incubated at the highest temperature (DuRant et al. 2013b), but DuRant et al. (2010, 2012a) reported no effect of incubation temperature on hatching mass. Tarsus length was always greater for ducklings incubated at the lowest temperature (DuRant et al. 2010, 2012a, 2013b). Taken together, these results suggest that prolonged development associated with low incubation temperature generates greater structural size in hatchlings at the expense of energy reserves.

Incubation temperature influences development of altricial species in a similar manner. Olson et al. (2006) incubated zebra finch (Taeniopygia guttata) eggs at
Wood ducks that were incubated at 35°C also ran and swam more slowly at 15 dph than ducklings incubated at the higher temperatures, but locomotor performance at 20 dph was no longer affected by incubation temperature (Hopkins et al. 2011).

In tree swallows, cooling of nests lengthened the incubation period and resulted in lower body mass and condition of nestlings up to 13 dph compared to nestlings from nests that were not cooled (Ardia et al. 2010). When tree swallow nests were heated, the body mass and condition of nestlings at 4 and 7 dph were greater than nestlings from unheated nests, but these differences disappeared for older nestlings (Pérez et al. 2008). By contrast, incubation temperature (35.0°C, 36.5°C, and 38.0°C) in blue tits did not affect body mass or wing length of nestlings at 2, 6, and 14 dph, but nestlings incubated at the lowest temperature had shorter tarsi at 14 dph (Nord and Nilsson 2011).

14.4.2 Metabolism and thermoregulatory ability

Relatively few studies have examined the effects of incubation temperature on metabolism and energy expenditure of avian embryos. In two species of megapodes, energy used by embryos increased 56% in Australian brush-turkeys and 77% in malleefowl (Leipoa ocellata) when incubation temperatures were reduced by 4°C (Booth and Jones 2002). In both species, low incubation temperature resulted in a longer ‘plateau phase’ of oxygen consumption at the end of incubation, which caused increased energy expenditure (Booth and Jones 2002).

Similarly, DuRant et al. (2011) incubated wood ducks eggs at 35.0°C, 35.9°C, and 37.0°C, and measured respiration every 3 days during incubation. They found embryos incubated at the highest temperature generally had the greatest daily metabolic rates and developed faster than embryos at lower temperatures, but embryos incubated at the lowest temperature expended more energy (120 kJ) over the course of incubation than embryos incubated at 35.9°C (83 kJ) and 37.0°C (94 kJ; Figure 14.2). These differences in energy expenditure occurred during the hatching process. Wood duck embryos incubated at the lowest temperature took 1–2 days longer to hatch once the eggshell was pipped than embryos incubated at higher temperatures (DuRant et al. 2011). Olson et al. (2006) reported zebra finch embryos from eggs that had been periodically cooled to 20°C during incubation had 14% higher metabolic rates at day 12 of incubation than embryos incubated constantly at 37.5°C. In blue tits, incubation temperature significantly influenced metabolic rates of nestlings.
The resting metabolic rate of nestling blue tits that had been incubated at 35.0°C was 7.5% and 8.1% higher at 14 dph than nestlings incubated at 36.5°C and 38.0°C, respectively (Nord and Nilsson 2011).

Experimental studies clearly show that incubation temperature has another important carry-over effect, that of influencing the development of thermoregulation (DuRant et al. 2011, 2012b). In one study, wood duck eggs were incubated at three temperatures (35.0°C, 35.9°C, and 37.0°C), and the thermoregulatory ability of recently hatched ducklings, i.e. 1 dph, was tested using a 1-hour thermal challenge at four temperatures (5°C, 10°C, 15°C, and 20°C; DuRant et al. 2011). Incubation temperature had no effect on a duckling’s ability to maintain body temperature during these thermal challenges, but larger ducklings were more effective at maintaining body temperature than smaller ones. However, in a second experiment a thermal challenge at 15°C showed that ducklings from the lowest (35.0°C) incubation temperature were only able to maintain their body temperature by spending 27% and 40% more energy than ducklings that were incubated at 35.9°C and 37.0°C, respectively (Figure 14.3a; DuRant et al. 2011). In another study, using the same three incubation temperatures, wood duck chicks were thermally challenged (10°C) at 1 dph and again at 4 dph to test improvement in their ability to thermoregulate (DuRant et al. 2013b). These ducklings were also assigned a restricted or an ad libitum diet to test effects of both pre- and post-hatch conditions on
the development of thermogenesis. Ducklings incubated at the highest temperature showed the greatest improvement in their ability to thermoregulate following the thermal challenge (Figure 14.3b). Importantly, improvement was influenced only by the pre-hatch conditions (incubation temperature) and not by post-hatch conditions (food availability; DuRant et al. 2013b).

### 14.4.3 Immune function

In several avian species, low incubation temperature slows development, and embryos use more energy and hatch with reduced body mass and nutrient reserves. Since immune activity is energetically expensive (Martin et al. 2003), low incubation temperature, because of its influence on the bioenergetics of development, may reduce the immune response of young birds. To examine this idea, DuRant et al. (2012a) incubated wood ducks eggs at 35.0°C, 35.9°C, and 37.0°C, and tested immunocompetence of ducklings at 6 dph using two novel antigens: phytohaemagglutinin (PHA) and sheep red blood cells (SRBCs). They found that the response to PHA and SRBCs was lower for ducklings incubated at the lowest temperature than the two higher temperatures (Figure 14.4). Tree swallows from nests that were cooled during incubation showed reduced innate immunity at 13 dph compared to nestlings from nests that had not been cooled (Ardia et al. 2010). Similarly, in 22 passerine species from Venezuela and Arizona, there was a strong positive effect of egg temperature on levels of natural antibody titres at hatching (Arriero et al. 2013).

Interspecific variation in intrinsic developmental rates of birds did not explain differences in the immune function of young birds without first accounting for variation in incubation temperatures (Martin et al. 2011). Taken together, the few studies that have examined the effect of incubation temperature on immune responses in birds indicate a positive relationship.

### 14.5 Effect of temperature-induced phenotypes on fitness

The environmental conditions experienced by birds during early development can have long-term effects on survival and reproduction (Lindström 1999; Monaghan 2008). We think it is unlikely that phenotypic changes induced by low incubation temperatures are adaptive in the majority of environmental contexts. For example, wood ducks incubated at low temperatures used more energy and hatched with reduced body mass and residual lipid reserves, and required more energy to thermoregulate than ducklings incubated at higher temperatures (DuRant et al. 2011, 2013b; Hepp and Kennamer 2012). Residual nutrient reserves are especially important to precocial young that must leave the nest soon after hatching, travel from nest sites to brood-rearing habitats, feed independently, and meet thermoregulatory challenges. Not surprisingly, mortality of precocial young is greatest within the first two weeks of hatching, and there is often a positive relationship between body mass of recently hatched ducklings and survival (Pelayo and Clark 2003; Traylor and Alisauskas 2006; Amundson and Arnold 2011). Hatching mass in birds generally has a positive effect on survival (Krist 2011).
Low incubation temperature also influences growth rates of young birds, which can have long-lasting effects. However, dispersal of young often makes it difficult to follow individuals to adulthood and first reproduction. In some species, like those of the Anatidae, strong natal philopatry of females makes it easier to examine effects of conditions experienced during early development on fitness. In brant geese (Branta bernicla), for example, body size of goslings in their first summer was influenced by early environmental conditions, i.e. food (Sedinger and Flint 1991), and body size of female goslings had positive effects on first-year survival and future fecundity (Sedinger et al. 1995, 2004). Furthermore, brant geese showed no evidence of compensatory growth; larger goslings became larger adults (Sedinger et al. 1995). Similarly, there was a positive relationship between first-year survival and body mass at fledging in female snow geese (Chen caerulescens), but the relationship was complex and occurred only when environmental conditions were constrained, i.e. during late hatching (Cooch 2002).

Immune responses and thermoregulatory abilities can be affected by incubation temperature and are generally weaker for individuals incubated at low temperatures. Robust immune responses are potentially important to young birds. In a meta-analysis of several species of birds, survival was greater for individuals with strong immune responses (Møller and Saino 2004). Nestling barn swallows (Hirundo rustica) with more robust immune responses relative to their brood mates also had increased survival and longevity (Saino et al. 2012). Similarly, normal development of homeothermy is critical for avian neonates. Energy expenses are high after hatching, especially in precocial species (Krijgsveld et al. 2012). Elevated energy demands by neonates to maintain homeothermy may slow early growth rate and tissue maturation, and affect their thermoregulatory ability (e.g. Rhymer 1988; Visser and Ricklefs 1995). Mortality is often greatest soon after young birds leave the nest, and hypothermia can be an important source of early mortality (Talent et al. 1983; Korschgen et al. 1996).

Trade-offs that influence the parents’ ability to provide an optimal thermal environment for developing embryos can compromise the phenotypic quality of their offspring in a number of important ways. It is likely that temperature-induced changes in offspring phenotype impact fitness of birds, much like they have been shown to influence that of reptiles (Warner and Shine 2008). However, studies linking incubation conditions of birds to fitness are still needed.

In the first study to test effects of incubation temperature experimentally on neonate survival and reproduction in a wild bird, Hepp and Kennamer (2012) artificially incubated wood duck eggs at three temperatures (35.0°C, 35.9°C, and 37.3°C) similar to those used by Hepp et al. (2006), DuRant et al. (2010, 2011, 2012a, 2012b, 2013b), and Hopkins et al. (2011). Recently hatched ducklings were individually marked, and broods were formed that contained ducklings from each of the incubation temperatures and placed in nestboxes of foster mothers. Since female wood ducks exhibit strong natal philopatry, recapture of females as breeding adults was used to test effects of incubation temperature on survival and recruitment to the breeding population. Reproductive success of these females also was monitored over four breeding seasons. Females incubated at 35°C were four times less likely to be recaptured than those incubated at higher temperatures. Females incubated at higher temperatures also had more than twice as many successful nests as those incubated at lower temperatures. This is the first study to show carry-over effects of low incubation temperature on survival, recruitment to the breeding population and subsequent reproductive success of birds.

14.6 Summary and future research perspectives

We have shown that incubation temperature influences the development of avian embryos and impacts an assortment of phenotypic traits of neonates that can carry over to later life stages. These effects are similar in many ways to those described for reptiles (Brown and Shine 2004; Deeming 2004b; Booth 2006; Mitchell et al. 2013; Du and Shine 2015). There are potential impacts on offspring survival and reproduction (Hepp and Kennamer 2012), so temperature-induced phenotypic effects in birds may play an important role in parental investment decisions, fitness, and the evolution of life histories (e.g. Monaghan and Nager 1997; Lyon and Eadie 2008; Martin 2008). However, further research is needed to test the strength, generality, and consequences of these relationships.

Experimental studies are needed to examine effects of incubation temperatures on hatching phenotypes and the mechanisms responsible for these changes (e.g. DuRant et al. 2014). We have identified some of these effects, but others may also exist. For example, neural development and cognitive performance are especially sensitive to conditions experienced during early development (Nowicki et al. 2002) and may be influenced
by incubation temperature (Amiel and Shine 2012). Furthermore, species may differ in how they respond to variation in incubation temperatures. We encourage studies that manipulate egg temperatures either by artificially incubating eggs for all or part of the incubation period or by manipulating egg temperatures in active nests (see DuRant et al. 2013a). These studies will be more meaningful if ecologically relevant egg temperatures are used.

Future research should also investigate whether effects of incubation temperature carry over to adulthood. We know that some temperature-induced effects can last for several days and weeks after hatching, but are these effects permanent or do they disappear? In some organisms, individuals that are stunted during early development can accelerate growth so that adult phenotypes are not compromised, but compensatory growth can be costly (Metcalfe and Monaghan 2001, 2003). Furthermore, some species show no evidence of compensatory growth (Sedinger et al. 1995). If phenotypic changes are relatively permanent, how do they affect fitness? Could conditions experienced during incubation help to alter an individual’s adult phenotype and be partly responsible for the variation in individual ‘quality’ that is so common in avian populations?

Finally, it will be essential to evaluate the influence of early developmental effects induced by incubation temperature on components of fitness. This can best be accomplished using an experimental approach (Hepp and Kennamer 2012) that creates different hatchling phenotypes by manipulating incubation temperatures, then marks individuals, cross-fosters them to remove effects of temperature from those of parental quality, and examines the effects of incubation temperature on survival and reproduction through long-term capture-mark-recapture studies. In many species of migratory passerines, the young disperse and do not return to natal areas as adults; therefore, not all species will be amenable to this type of long-term experimental study. However, in many species, like waterfowl and non-migratory passerines, individuals show high levels of natal philopatry and breeding-site fidelity and can be monitored as breeding adults (Weatherhead and Forbes 1994; Hepp and Kennamer 2012). Future investigations such as these will help to clarify the importance of incubation temperature on development of avian neonates and the significance of investment decisions made by incubating parents on overall reproductive success.

Acknowledgements

We thank our field and lab crews, Bobby Kennamer, and the staff at the University of Georgia’s Savannah River Ecology Laboratory. We also thank Charles Deeming for his editorial assistance. Our research was supported by National Science Foundation (NSF) grant IOB-0615361 to GRH and WAH, NSF Doctoral Dissertation Improvement Grant DEB-1110386 to SED, the Alabama Agricultural Experiment Station, Fralin Life Science Institute, and the U.S. Department of Energy under award DE-FC09-07SR22506 to the University of Georgia Research Foundation.